University of Alberta

Studies on Benzo(thio)pyrano[2,3-b]pyridines.

by

Jane Anne Nagel

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in

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Facultyof Pharmacy and Pharmaceutical Sciences

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled Studies on Benzo(thio)pyrano[2,3-b]pyridines in partial fulfilment of the requirements for the degree of Master of Science in Pharmaceutical Sciences (Medicinal Chemistry).

Dr. F.M. Pasutto

Dr. G.B. Baker

Dr. R.T. Coutts

May 14, 1996

to my friends and family

Abstract:

Recent research has demonstrated an important role for the products of the 5-lipoxygenase pathway of the arachidonic acid cascade in asthmatic conditions. Previous researchers in our laboratory found the benzo(thio)pyrano[2,3-b]pyridine system to have an effect on broncho-constriction, but the mechanism of action was not fully established. Potassium channel agonists, exemplified by pinacidil and cromakalim, with guanidinyl and nitroethyl side chains, are an emerging group of compounds with potential for anti-asthmatic action. A series of reactions attempted to synthesise a furo[2,3-b]chromone ring system and a number of C-7 substituted benzo(thio)pyrano[2,3-b]pyridines. Initial target molecules of C-7 substituted benzo(thio)pyranopyridines with guanidinyl and nitroethyl side chains were not realised, nor was the ring closure of the furochromones. A facile reaction was devised to produce C-7 substituted diazamino side chains including dimethyl-, diethyl-, dipropyl-, di-isopropyl-, morpholino-, and pyrrolidino- benzo(thio)pyranopyridines. Additions of the aromatic amines diphenylamine and dibenzylamine as well as the addition of N-benzylpiperazine to the 7-diaza benzo(thio)pyranopyridines were also unsuccessful.

Acknowledgement:

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LIST OF ABBREBYTIONS:

AA	arachidonic acid
amu	atomic mass units
ATP	adenosine triphosphate
°C	degrees Celsius
^{13}C NMR	carbon-13 nuclear magnetic resonance spectroscopy
$CDCl_3$	deuterated chloroform
CI-MS	chemical ionisation mass spectroscopy
cm^{-1}	inverse centimetres
CO	cyclo-oxygenase
d	doublet
dd	doublet of doublets
DMF	dimethylformamide
DMSO-d_6	hexadeuterodimethylsulfoxide
dt	doublet of triplets
EI-MS	electron impact mass spectroscopy
FLAP	5-lipoxygenase activating protein
g	gram
^{1}H NMR	proton nuclear magnetic resonance spectroscopy

5-HETE	5-hydroxyeicosatetraeneoic acid
5-HPETE	5-hydroperoxyeicosatetraeneoic acid
Hz	hertz
IL-2	interleukin-2
IL-6	interleukin-6
IR	infrared spectroscopy
J	coupling constant
KCA	potassium channel agonist
LO	5-lipoxygenase
LT	leukotriene
LTA_4	leukotriene-A_4
LTB_4	leukotriene-B_4
LTC_4	leukotriene-C_4
LTD_4	leukotriene-D_4
LTE_4	leukotriene-E_4
M	molecular ion
m	multiplet
$M^{+\cdot}$	molecular ion
m/z	mass to charge ratio, z assumed to be equal to 1
mg	milligram

MHz	megahertz
mL	millilitre
mmol	millimole
nM	nanomolar
PPA	polyphosphoric acid
precipitate	precipitate
pS	picofiemens
psi	pounds per square inch
q	quartet
s	singlet
t	triplet
td	triplet of doublets
THF	tetrahydrofuran
TLC	thin layer chromatography
TNFα	tissue necrosis factor α
TXB_2	thromboxane B_2
wt.	weight

INTRODUCTION:

1.0 Introduction to asthma as a chronic inflammatory disease:

Asthma is a chronic condition of inflammation and reversible constriction of the bronchial tree caused by either extrinsic factors such as environmental allergens or aggravating factors, such as pollen, or intrinsic factors, such as exercise-induced asthma. The features of inflammation and excess mucus production are mediated by a wide variety of endogenous compounds including leukotrienes B_4, C_4, D_4, and E_4 (Gleason *et al.*, 1986; Larsen and Acosta, 1993), tissue necrosis factor-α (TNFα), interleukin-2 (IL-2) and interleukin-6 (IL-6). The bronchoconstriction is also linked to potassium channels. Inhibition of any of these factors should play a role in altering the course of this disease by either preventing an attack from occurring, or stopping an attack once it is already in progress.

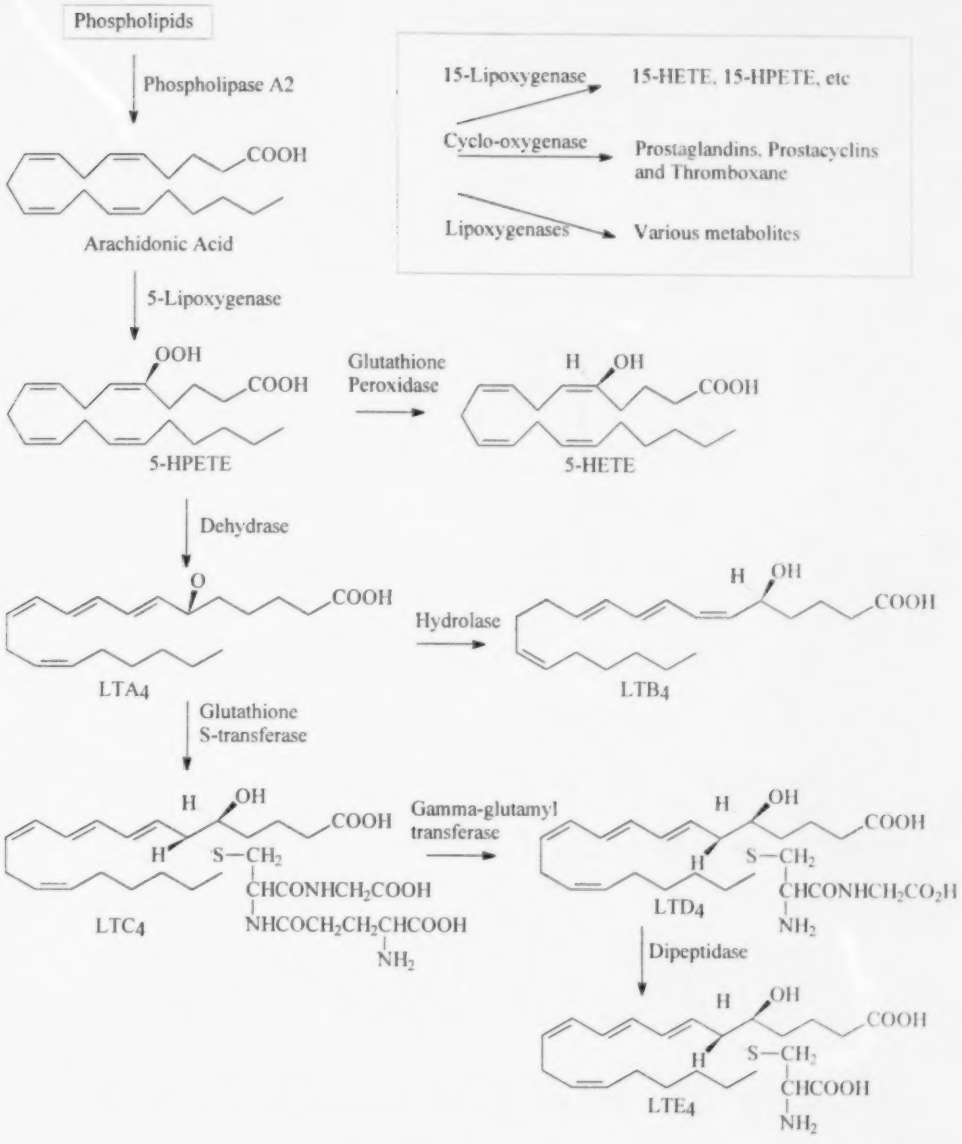
1.1 Altering the course of asthma by LT interference:

Inhibition and antagonism of the leukotrienes (LTs) have been attempted for several years with few clear successes. It has been discovered that the interaction of these compounds in the lungs is far more complex than initially expected thus leading to the development of a wide variety of seemingly unrelated medicinal compounds all attempting to produce the same results (McMillan, 1993). In order to affect the LTs, one must first look at their action. Leukotrienes are products of the arachidonic acid (AA) cascade and are produced by the action of 5-lipoxygenase (5-LO) on the

arachidonic acid found in the phospholipid bilayer of most cells (Needleman *et al.*, 1986). Leukotrienes formed through the action of 5-LO encompass one arm of the AA cascade. The other important arm of the AA cascade involves the metabolism of AA by cyclo-oxygenase (CO) to form the prostaglandins, thromboxanes, and prostacyclines (Needleman *et al.*, 1986). It is important to remember that there are several other arms to this cascade but the large scope entailed does not allow for their discussion here (Needleman *et al.*, 1986).

1.2 Role of LTs in inflammatory conditions:

Leukotrienes are known to play a role in diseases of inflammation such as asthma, ulcerative colitis, psoriasis, and rheumatoid arthritis. The LTs most responsible for damage include LTB_4, and LTD_4 which, along with LTC_4 and LTE_4, were previously known as the slow reacting substance of anaphylaxis (SRS-A) (Larsen and Acosta, 1993). Leukotrienes C_4, D_4, and E_4 are also referred to as the peptido-leukotrienes. Leukotriene B_4 is an inflammatory factor as well as a powerful chemotactic agent for a variety of leukocytes and can induce edema. Leukotriene B_4 is primarily produced in the polymorphonuclear leukocytes whereas the peptido-LTs are synthesised mainly in mast cells (McMillan, 1993). Leukotriene D_4 has been shown to be a factor in inflammation, bronchoconstriction, and mucus production.



5-Lipoxygenase pathway to produce the leukotrienes from phospholipids. Adapted from J.S. Larsen and A.P. Acosta, 1993.

1.3 Production of LTs:

Arachidonic acid is acted upon by 5-LO in a series of steps to produce the LTs (Musser and Kreft, 1985). Initially, the 5-LO enzyme, which at rest is located in the cytosol, must be translocated to the cell membrane so that it can interact with the released AA. The enzyme is thought to be a non-haem iron-containing enzyme which goes through a Ca^{++}, ATP, (Musser and Kreft, 1992; Ford-Hutchinson, 1993) and Fe^{3+} dependent reaction to become bound to a transmembrane protein known as Five-Lipoxygenase Activating Protein (FLAP). It is then able to place an oxygen at the 5 position of AA. The exact mechanism of this reaction is, however, still not fully understood (McMillan and Walker, 1992).

1.4 Opportunity for LT synthesis inhibition and LT antagonism:

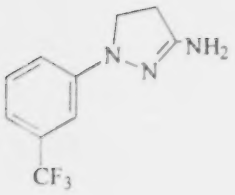
The sequence involved in the initial metabolism of AA to the LTs allows for three good sites of intervention in order to block this process and to prevent the formation of the inflammatory processes caused by the various LTs. The first site is interference with the red-ox reaction involved in activating the movement of the 5-LO to the FLAP (McMillan and Walker, 1992). Molecules which can change the red-ox potential can block this first step. The second area of interaction can be at the FLAP binding site of 5-LO. Molecules which can mimic 5-LO can compete for binding sites to prevent 5-LO's action on the AA (McMillan, 1993; Young *et al.*, 1993). Finally, the third area of interaction can be blockade of the AA site on FLAP (McMillan, 1993). Molecules which can mimic the structure of AA will lead to a decreased amount being metabolised to LTs. The other manner in which the actions of LTs can

be modified is, of course, by antagonism at the site of action of the LTs (von Sprecher *et al.*, 1993).

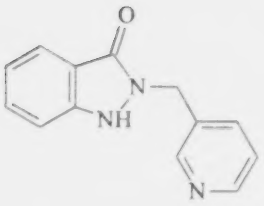
2.0 COMPOUNDS WITH LT INHIBITORY POTENTIAL:

2.1 Inhibition of red-ox reaction:

Initially, inhibition of the red-ox reaction was thought to be an excellent choice for affecting the production of the various LTs. Unfortunately, since there is a multitude of red-ox reactions occurring in the body the potential for deleterious side effects was high. Although the red-ox potential for the reaction involved in the iron containing non-haem enzyme is different from that in other areas, the likelihood of developing a medicinal compound with sufficiently selective red-ox inhibitory potential was not practical. Nonetheless, three important series of compounds were synthesised in this category: the dual inhibitors of cyclo-oxygenase and 5-LO such as BW-755C (McMillan and Walker, 1992), the substituted indazolines such as ICI-207 986 (McMillan and Walker, 1992), and substituted naphthols such as DUP-654 (Batt *et al.*, 1990). This line of red-ox inhibitors, however, has been largely abandoned due to the common problem of interference in the haemoglobin red-ox reaction causing methaemoglobineamia (Edwards *et al* and McMillan, 1991).



BW-755C



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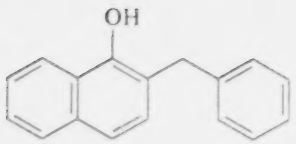
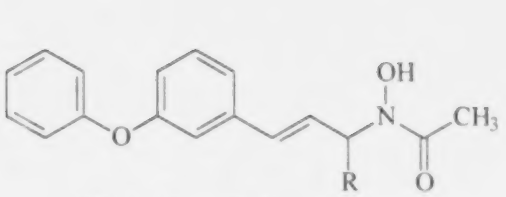


DUP-654

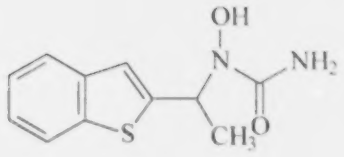
Potential inhibitors of non-haem 5-LO enzyme red-ox reactions.

2.2 Chelating hydroxamates and N-hydroxyureas:

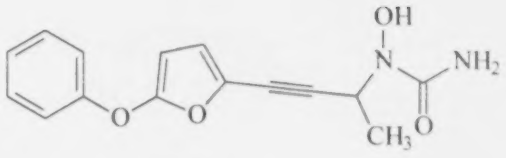
The hydroxamic acids and N-hydroxyureas have, in recent years, been the most promising compounds for inhibiting the 5-LO conversion of AA to the LTs. These compounds act as chelators of the Fe^{3+} necessary for the function of 5-LO. Most are based on the template of AA: they are intended to be analogs of AA which will allow the 5-LO to recognise them as substrates and, subsequently, allow the compounds to chelate the Fe^{3+}, preventing any further reaction of the 5-LO. Examples of compounds in this class include: zileuton, formerly A-64 077 (Hsiao and Kolasa, 1992; Musser and Kreft, 1992; Satoh *et al.*, 1993), A-78 773 (Bell *et al.*, 1993), and BW-A4C (Musser and Kreft, 1992).



BW-A4C R=H
BW-B218C R=CH_3



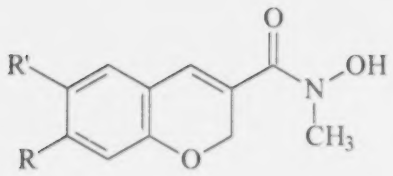
zileuton (A-64 077)



A-78 773

Promising hydroxamic acids and N-hydroxyureas for inhibition of 5-LO.

Zileuton was the first important discovery in this class and has demonstrated its ability to prevent production of leukotrienes in biological systems challenged with allergens (Hsiao and Kolasa, 1992). The hydroxamic acid A-78 773 was found to be more potent than zileuton and did not show evidence of shunting of AA metabolites to the CO pathway (Bell *et al.*, 1993) as no increase in TXB_2 was observed in studies. Hydroxamic acids of the chromenes explored by Satoh *et al.* were determined to be comparable in potency and function to zileuton (Satoh *et al.*, 1993). Increasing the lipophilicity of the chromenes enabled these researchers to increase the potency of their test compounds, as has been demonstrated for other 5-LO inhibitors such as the red-ox inhibitor DUP-654 (Batt *et al.*, 1990).

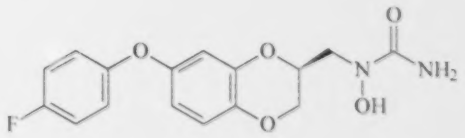


R = H or $PhCH_2O$; R' = H or CH_3

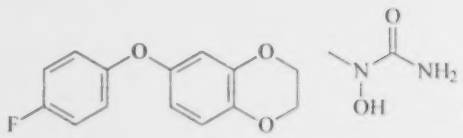
Representative chromenes explored by Satoh *et al.* (1993).

Similar to zileuton, BW-A4C shows good selectivity for 5-LO over CO (about 20 times more selective for 5-LO over CO), but the bronchial relaxation effect of these compounds does not seem to correlate well with their power to inhibit 5-LO. This inconsistency suggests that another mechanism is at play and one cannot confidently correlate a new compound's 5-LO inhibitory power with its potential to be a useful bronchodilator in the treatment of asthma (McMillan and Walker, 1992). Again, structure-activity relationships of these compounds cannot be accurately determined as the compounds seem to interact with the 5-LO enzyme in a non-specific manner and lipophilicity seems to be the dominant factor: enantiomers of zileuton and BW-B218C have identical *in vitro* activity (McMillan and Walker, 1992; Greco *et al.* 1992; Ohemeng *et al.*, 1994). It is important to note, however, that indefinite increases in the lipophilic nature of the hydroxamic acids eventually result in decreased *in vivo* activity due to poor oral availability when the lipophilic nature reaches a maximum tolerated value (Ohemeng *et al.*, 1994). Evidence is also increasing to support the theory that the red-ox potential of these hydroxamic acids may be playing a role in their activity and thus their chelating ability may not be their dominant property. Their utility *in vivo* may be limited for the same reasons as the

red-ox inhibitors (McMillan and Walker, 1992). The recently reported chromene compound CGS-25 997 and its dextro-rotamer CGS-25 998 have demonstrated enantioselectivity in *in vitro* guinea pig polymorphonuclear 5-LO assays and in *ex vivo* dog models which have challenged earlier observations that this class of compounds was interacting in a non-specific manner (Satoh *et al.*, 1995). Potency did increase along the same lines as the other compounds [increasing lipophilicity lead to increased *in vitro* activity (Satoh *et al.*, 1993)], but the *2S* enantiomer was considerably more potent than the *2R* enantiomer when administered intravenously or orally in the *ex vivo* studies (Satoh *et al.*, 1995).



2*S*-(+)-enantiomer CGS-25 997



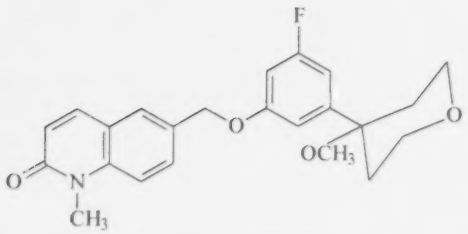
2*R*-(-)-enantiomer CGS-25 998

Hydroxamic acids which show enantioselective pharmacodynamics.

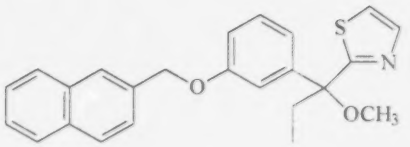
2.3 Non-red-ox inhibitors:

Since few of the iron ligand or red-ox inhibitors react with 5-LO with much specificity (i.e. lipophilicity was the dominant factor in activity), and thus have a greater potential for side effects, other types of inhibitors have been explored, including the general category of non-red-ox inhibitors. The non-red-ox type inhibitors are exemplified by the methoxyalkylthiazoles ICI-211 965, ICI-216 800,

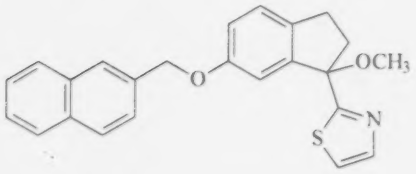
(McMillan and Walker, 1992) and methoxytetrahydropyrans such as ICI-D2 138 (also known as ZD-2138) (Crawley *et al.*, 1992; Crawley *et al.*, 1993; Foster *et al*, 1994).



ICI-D2 138 (ZD-2138)



ICI-211 965



ICI-216 800

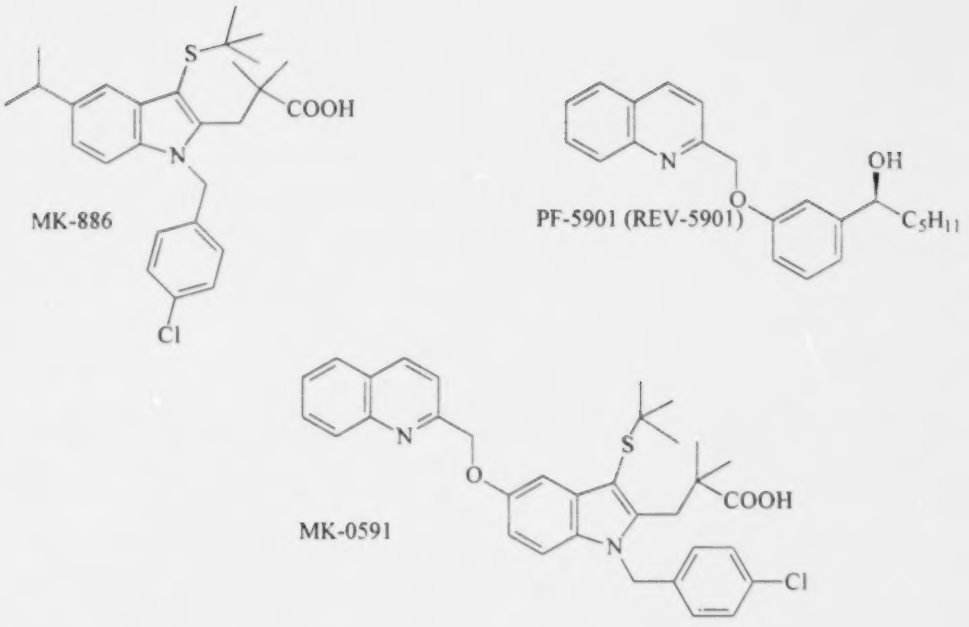
Methoxyalkylthiazoles and methoxytetrahydropyran non-red-ox inhibitors of 5-LO.

The early methoxyalkylthiazoles were not orally active due, in large part, to their poor aqueous solubility. Compound ICI-216 800 had better availability than ICI-211 965, but their short half-lives limited their potential as inhibitory drugs (McMillan and Walker, 1992). These compounds did, however, show enantioselectivity in potency and were thus regarded as useful lead compounds (Crawley *et al.*, 1993). The development of ZD-2138 and several derivatives of the methoxyalkyl thiazole and methoxytetrahydropyran classes followed, providing further proof of enantiomer potency differences (Foster *et al*, 1994). Inhibition of the

CO pathway has not been noted with ZD-2138 and its derivatives and ZD-2138 has been demonstrated to be at least 10 times more potent than zileuton in bronchoconstriction models (Foster *et al*, 1994). Thus, ZD-2138 has become one of the more promising compounds in this area and is currently undergoing clinical testing (McMillan and Walker, 1992; Foster *et al*, 1994).

2.4 Inhibitors of translocation (interference in the interaction between FLAP and 5-LO):

Recognition of the inhibitors of the interaction between FLAP and 5-LO began with MK-886 as it was an effective inhibitor of LT biosynthesis in intact leukocytes but was ineffective in cell-free isolates of 5-LO (Prasit *et al.*, 1993). Derivatives of MK-886 (Young *et al.*, 1993), such as MK-0591 (Vickers *et al.*, 1993), REV-5901, now called PF-5901, (Musser and Kreft, 1992), and WY-50 295 (Musser and Kreft, 1992), have shown promise in asthma treatment as well as inflammatory bowel disease.



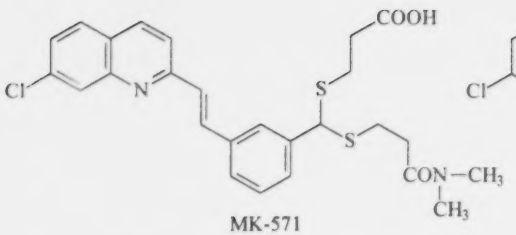
Translocation inhibitors.

The development of this class of 5-LO inhibitors closely followed that of the LT antagonists. They may have a dual action of inhibition, i.e. FLAP and LT antagonistic activity as exemplified by PF-5901 (Musser and Kreft, 1992), or they may be singularly FLAP inhibitors such as MK-886 (Prasit *et al.*, 1993). As mentioned above, MK-886 has no affect on free 5-LO enzyme but can prevent the synthesis of LTs in intact cells. The compound has a specific interaction with the FLAP as was demonstrated by the variations in potency when molecular modifications were made to this lead compound (Prasit *et al.*, 1993; Young *et al.*, 1993).

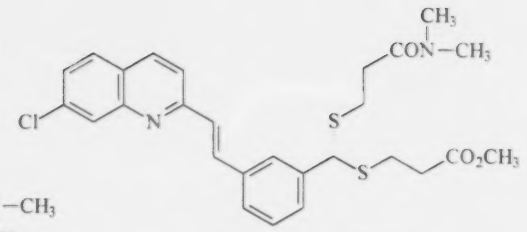
3.0 ANTAGONISTS OF LEUKOTRIENES:

Because LTD_4 and LTB_4 were considered to play the most significant role in the disease process of asthma, antagonists of these two peptido-leukotrienes were considered to be the most promising. As described previously, compounds such as MK-886 were initially thought to be LT receptor antagonists but later proved to be LT synthesis inhibitors. As other drugs which interfere in the FLAP-LO interaction were also found to be site antagonists, new developments were spawned by alterations in their structures. Compounds such as MK-571 (Young *et al.*, 1993), MK-679, also called verlukast (Ford-Hutchinson, 1993), ICI-204 219 (Brown *et al.*, 1992; Jacobs *et al.*, 1993), LY-171 883 (von Specher *et al.*, 1993), RG-12 525 (Musser and Kreft, 1992), are all examples of such modifications, whereas the development of SKF-104 353 (Harper *et al.*, 1992) was based on designing a compound specifically from the receptor structure.

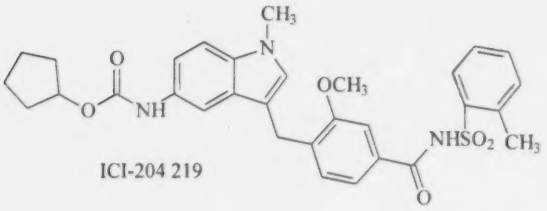
Clinical testing of MK-679 [(*R*)-verlukast] was promising and proceeded to phase-two clinical trials but at that point it was discovered that this compound caused increases in liver enzymes and was hepatotoxic. Further testing was abandoned (Young, lecture 1992). Currently, despite early successes, antagonists of the LTs have not as yet been introduced into the market.



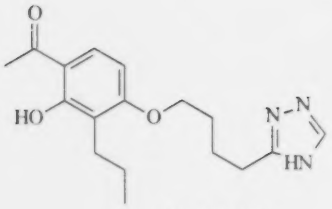
MK-571



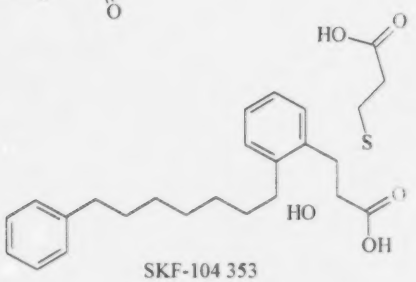
MK-679 (verlukast)



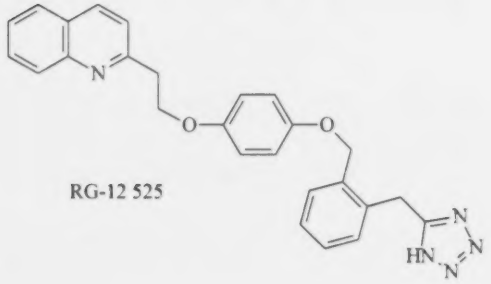
ICI-204 219



LY-171 883



SKF-104 353



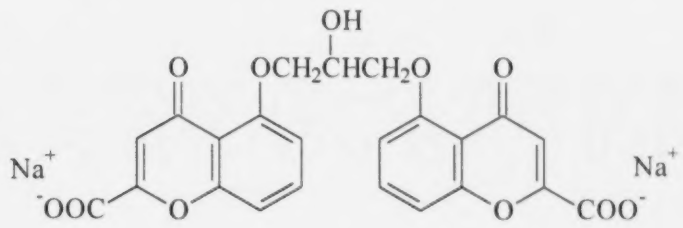
RG-12 525

Various antagonists of LTs.

4.0 PREVIOUS WORK FROM OUR LABORATORY IN THE SEARCH FOR LT RECEPTOR ANTAGONISTS AND/OR LT SYNTHESIS INHIBITORS:

4.1 Benzopyrano[2,3-b]pyridines in the literature:

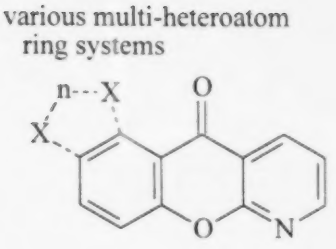
A search of recent literature was not fruitful in obtaining many examples of benzopyrano[2,3-b]pyridines as LT synthesis inhibitors or as LT antagonists. Nohara *et al.* (1985) gave the best examples of benzopyrano[2,3-b]pyridines as anti-allergic and anti-anaphylactic agents. The development of these compounds was based on the model of disodium cromoglycate (a marketed bronchodilator) and the recognition of the anti-allergic properties of this di-benzopyran ring system.



Disodium cromoglycate

4.2 Research on benzopyrano[2,3-b]pyridines in our laboratory:

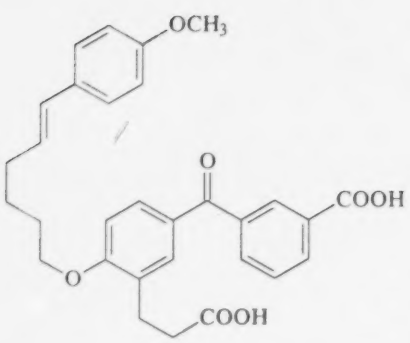
Previous researchers in our laboratory found some success in initial *in vitro* testing but no further development had occurred before the project at hand. Numerous compounds were synthesised and tested *in vitro* on guinea pig isolated tracheal strips and lung parenchyma strips which had been challenged with AA to evaluate their ability to reverse contractions produced. Indomethacin (a known CO inhibitor) was added in order to isolate the 5-LO metabolites from those produced from the CO pathway. Other arms of the AA cascade were not investigated. In this way investigators were able to deduce that the contractions produced were due to products of the 5-LO pathway rather than by metabolites of the CO pathway. Reversal of contraction produced upon addition of the test compounds was indicative of 5-LO pathway inhibition but was not specific as to the mechanism of inhibition (Vudathala, 1990). Compounds which showed airways smooth muscle relaxing activity were further tested in histamine challenge studies and comparison studies to known LT antagonists. Although concentration-response curves were established for the histamine studies, a definite correlation to LT antagonist activity could not be determined (Vudathala, 1990). At this point, further research on these compounds was to be handled by Eli Lilly, as a non-disclosure agreement was signed and compounds were submitted to them for testing.



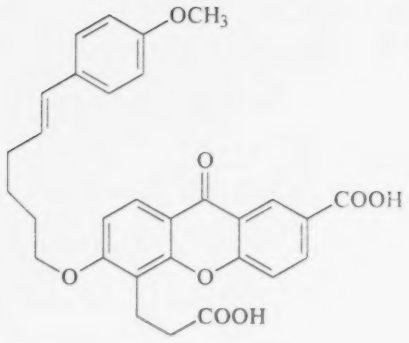
Variations on benzopyrano[2,3-b]pyridine nucleus.

4.3 Xanthones as LT antagonists:

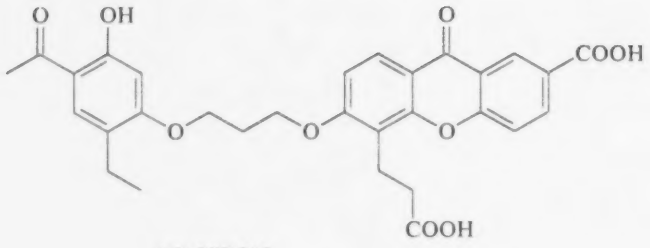
The related compounds, the xanthones, have also seen limited exploration for use as LT antagonists (Sawyer *et al.*, 1993). Researchers with Eli Lilly have discovered the importance of this ring structure as well as the need for the large lipophilic tail region for good antagonist activity of LTB_4. Compound LY-282 210 and the related LY-210 073 and LY-223 982 are good examples of this class.



LY-223 982



LY-210 073



LY-282 210

Xanthone type LTB_4 antagonists developed by Eli Lilly.

4.4 Benzopyrano[2,3-b]pyridines as potential potassium channel agonists (KCAs):

During *in vitro* investigation of the compounds produced previously in our laboratory, it was noted that some compounds were not only producing inhibition to broncho-constricting agents, but also showed some intrinsic broncho-relaxing activity. Although the exact mechanism of this broncho-relaxation was not established, it was supposed that these compounds were not acting as LT antagonists (Vudathala, 1990). At this point it was reasonable to suggest that the activity might be

due to an action on the potassium channels present in the guinea pig bronchial strips. Further testing on these compounds, was, however, not pursued at that time.

5.0 POTASSIUM CHANNELS:

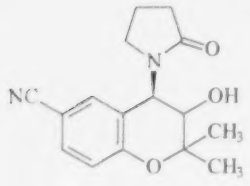
5.1 Potassium channel presence and function:

Potassium channels are essential for repolarisation and maintenance of resting potentials of smooth and skeletal muscle cells. Initially interest was focused on the potential for these compounds as antihypertensive agents but their utility in heart ischemia reversal, angina pectoris, peripheral artery disease (Lenz and Wagner, 1995), urinary incontinence, epilepsy, irritable bowel syndrome and bronchial asthma (Evans and Taylor, 1994) has been recently investigated.

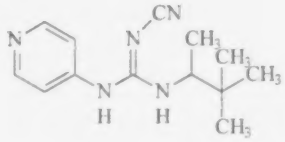
5.2 Therapeutic uses of potassium channel agonists:

Inhibition of potassium channels has been a well explored area in the past, with compounds such as glyburide in the treatment of non-insulin dependent diabetes mellitus (Lenz and Wagner, 1995), but it is only in more recent years that the potential for a therapeutic use for agonists or openers has been explored. With the realisation that the diuretic diazoxide was prone to inducing hyperglycaemia in patients due to the opening of potassium channels on the β-pancreatic cells, the exploration of potassium channel agonists (KCAs) began. Cromakalim and pinacidil

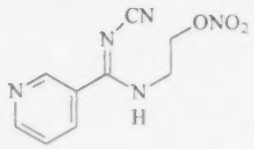
are widely cited as the first true KCAs, with nicorandil also frequently cited. The difference between cromakalim and pinacidil *versus* nicorandil is that nicorandil's activity is partly due to its similarity to the nitrate family of antihypertensive drugs (Lenz and Wagner, 1995). Nicorandil is thus not a true KCA but rather a hybrid of KCA and a nitrate-type antihypertensive compound.



cromakalim



pinacidil



nicorandil

Earliest examples of potassium channel agonists.

5.3 Classification of potassium channels:

As research proceeded into the field of KCAs, it was discovered that a large number of different potassium channels existed. These channels can be broken down into three major types with various sub-types which are well described, however up to ten major classes may exist. The calcium activated potassium channels were among the first identified and are divided into three sub-types: high conductance (100-250 pS where pS is picofiemens, a unit for physical conductance); intermediate conductance (18-50 pS); and low conductance (10-14 pS) (Atwal *et al.*, 1992). These channels are involved in a wide variety of tissues, from the neurones, cardiac cells and some smooth muscle to the red blood cells and other smooth muscle cells (Atwal *et al.*,

1992). As the channels are opened by depolarisation, they allow intracellular levels of calcium to increase, thus aiding in cell repolarisation. It has been suggested that cromakalim may have some effect on the large conductance channels but this is still inconclusive (Wickenden, 1991).

The second major type of potassium channel includes the voltage dependent potassium channels. This type can also be divided into subgroups: delayed rectifier current (10-50 pS); inward rectifier current (5-30 pS); and transient outward current (20 pS) (Lenz and Wagner, 1995). The discovery and classification of this channel type has been a recent advancement and although not much is known, these channels are thought to be mostly responsible for repolarisation of cardiac and non-cardiac action potentials (Atwal *et al.*, 1992).

The most important potassium channels for our purposes are the metabolically gated channels. These channels are thought to be the ones most affected by the actions of the currently evolving KCAs. The metabolically gated channels can be sub-typed into ATP sensitive channels, arachidonic acid/fatty acid modulated channels, and acetylcholine activated channels (Atwal *et al.*, 1992; Lenz and Wagner, 1995).

Calcium (Ca^{2+})-activated K^+ channels
Large (100-300 pS) conductance channel
Intermediate (15-30 pS) conductance channel
Small (10-14 pS) conductance channel
Voltage-dependent K^+ channels
Delayed rectifier current (conductance 10-50 pS)
Inward rectifier current (conductance 5-30 pS)
Transient outward current (conductance 20 pS)
Metabolically gated K^+ channels
ATP-sensitive K^+ channels
Arachidonic acid/fatty acid-modulated K^+ channel
Acetylcholine-activated K^+ channel

Types and sub-types of potassium channels. Adapted from Lenz and Wagner, 1995.

5.4 Therapeutic utility of KCAs in asthma:

Several different types of channels may be present on any given cell and thus types are not limited to certain tissues (Lenz and Wagner, 1995). As a result it becomes more difficult to direct the activity of a pharmaceutical to one specific tissue such as the airways or the heart. Although compounds such as cromakalim do show effects on the bronchi of asthmatic patients, without adversely affecting blood pressure or heart rate in some studies (Anonymous, 1989), other reports describe poor selectivity for the lungs (Gopalakrishnan *et al.*, 1993).

Since the late 1980s, the role of KCAs has been focused largely on cardiac and vasculature activities; however, use of these compounds in asthma disease

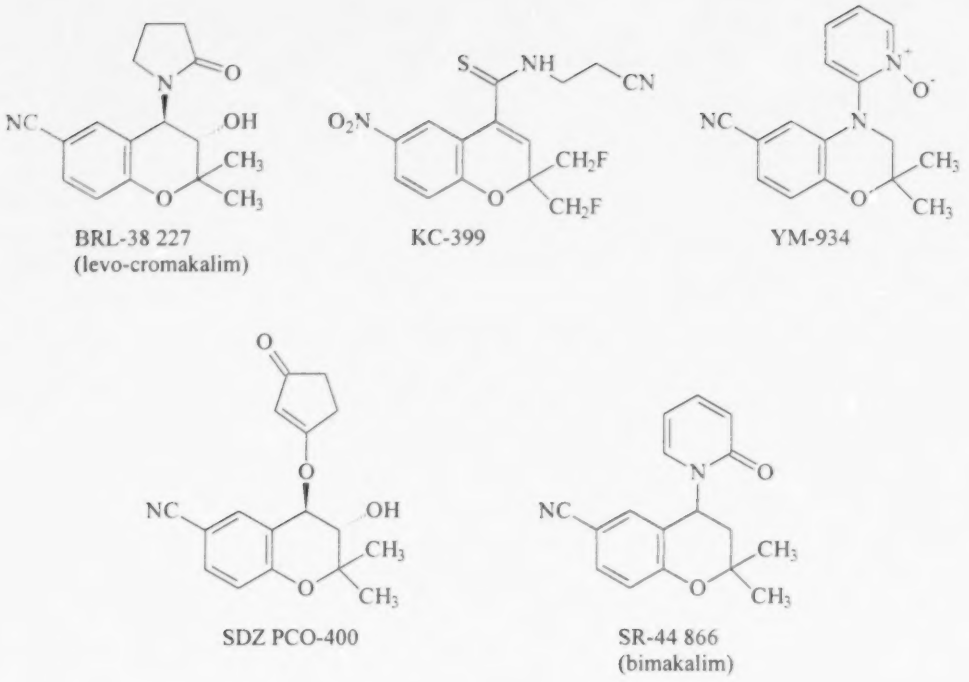
modification and use in chronic obstructive pulmonary disease continues to develop albeit with less research emphasis than on their cardiac role. Cromakalim and pinacidil were the first of this class to be evaluated in asthmatic patients and the results were initially promising (Anonymous, 1989).

5.5 Categorisation of KCAs based on chemical structure:

The KCA compounds can be divided into three general structural categories: the benzopyrans such as cromakalim, the pyridines such as pinacidil, and the thioformamides. Although there are other categories such as the pyrimidines and the benzothiazines, represented by minoxidil and diazoxide respectively, these compounds are not well established as true or pure KCAs (Lenz and Wagner, 1995) and as such will not be discussed at this time.

5.5.1 Benzopyran class of KCAs:

The benzopyran class is the most explored and contains compounds with airways activity such as cromakalim (BRL-34 915) (Hamilton and Weston, 1986), levo-cromakalim (BRL-38 227) (Evans and Taylor, 1994; Taylor *et al.*, 1992), bimakalim (EMD-52 692 or SR-44 866) (Lenz and Wagner, 1995), SDZ PCO-400 (Evans and Taylor, 1994), KC-399 (Pirotte *et al.*, 1995), and YM-934 (Evans and Taylor, 1994; Pirotte *et al.*, 1995).



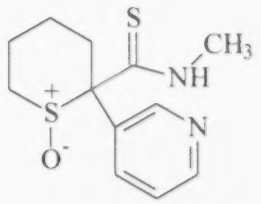
Benzopyran series of KCAs active on airways smooth muscle.

Studies on the activity of cromakalim and levo-cromakalim have shown that there is likely more than one explanation for the activity of these compounds. Although early studies of cromakalim revealed that a concentration of 100 nM was enough to fully suppress spontaneous activity in rat portal vein (Hamilton and Weston, 1989), it was later noticed that concentrations of three to five times that number induced a vasorelaxation (Quast, 1993). Studies on a wide variety of cardioactive KCAs also revealed this tendency to induce further relaxation with doses above those required for suppression of spontaneous activity despite the fact that they were from widely varied structural types (Bray and Quast, 1992). From these reports,

it was proposed that there was another mechanism of action for these compounds and likely they were influencing intracellular levels of ATP, calcium stores, and other cellular mediators (Quast, 1993). These studies lead to the categorisation of the potassium channels as outlined above.

5.5.2 Thioformamide KCA compounds:

The next group, the thioformamides, contains only one important example of a compound with airways activity, RP-49 356 (Lenz and Wagner, 1995), although it is important to note that several cardioactive compounds fit into this category.



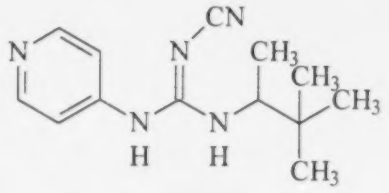
RP-49 356

Thioformamide KCA with airways activity.

5.5.3 Pyridine KCA compounds:

The pyridine group contains only one compound, pinacidil, with known activity on airways. It is again important to note that there are several cardioactive

compounds which fit into this category, but the only one with known airways-activity is pinacidil.



pinacidil

Pyridine compound with airways activity.

The structure activity relationship for the pyridinyl compounds has been extensively studied by Manley and Quast (1992). They were able to find a good correlation between the specific conformation and structure elements of pinacidil and its derivatives which were cardioactive but demonstrated no activity in airways. It was shown that the aryl group, such as the pyridine in pinacidil, must be substituted at the 3-position in order to obtain good cardiac activity as derivatives with a 4-substituent on the pyridine showed poor or no cardiac activity in their *in vitro* studies (Manley and Quast, 1992). They also found that the cyanoguanidine, nitroethyl and thiourea substituents were favourable, but urea, although very close in structure and C-N bond lengths, was not effective. Manley and Quast have attributed this to the conformations available to the cyanoguanidine, nitroethyl and thiourea groups but not the urea group. These studies show that, unlike the majority of the leukotriene synthesis inhibitors and leukotriene antagonists, KCAs show a definite receptor interaction in order for these compounds to demonstrate significant activity.

OBJECTIVES OF RESEARCH:

The detection of LTs in bronchial lavage fluid of asthmatic patients has focused awareness of the importance of these mediators in asthma. The course of disease may be altered by inhibiting the action or the synthesis of the various leukotrienes. Previous research done in our laboratory has shown that the benzopyrano[2,3-b]pyridines have the potential to reverse bronchoconstriction and may also posses the ability to cause bronchodilation.

From the previous work in our laboratory, we intended to synthesise further analogs of the benzopyrano[2,3-b]pyridines to continue the exploration of their utility. Keeping in mind the unknown mechanism of bronchodilation of some of the previously produced compounds, attention was also turned to the emerging role of potassium channel agonists. Compounds such as cromakalim and pinacidil offered examples of known KCA entities and thus were used as leads for our target molecules. It was felt that the guanidinyl and nitroethyl side chains were essential for activity of these compounds, and attachment of these moieties to the benzopyrano[2,3-b] pyridine parent ring system was the initial goal of our research.

PROJECT OVERVIEW:

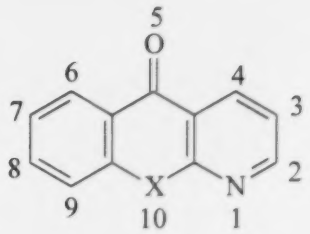
Initially, work in our laboratory was focused on creating new hydroxamic acids utilising the benzopyrano[2,3-b]pyridine and furo[2,3-b]chromone parent molecules. Both the benzopyranopyridine and furochromone parent tricyclic ring structures were similar in conformation to the compounds presented in the previous section and also met the criterion for hydroxamic acids as they were similar to the AA back bone. It was proposed that our projected targets would have potential activity as they logically fit into this category. Unfortunately, due to significant synthetic barriers encountered in the synthesis of both series, this area was abandoned. Work in our laboratory done by previous researchers pointed to benzopyrano[2,3-b]pyridines as potential potassium channel agonists, which led us to proceed to this emerging area. Increasingly, the role of potassium channels in the bronchoconstriction component of asthma has been recognised and affecting their function has become a focal point for the control of this disease.

The initial target molecules for this thesis were C-7 substituted benzopyranopyridines (and benzothiopyranopyridines) containing the guanidinyl, thioguanidinyl, and nitroethyl functional groups. The progress was very poor and ultimately this series of compounds was abandoned. A search for a new parent system resulted in the attempt to use a furochromone as the new lead compound. The furo[2,3-b]chromone was selected for the similarity of its structure to the benzopyrano[2,3-b]pyridines and also based on the fact that it was an electron-rich tri-cyclic ring system (unlike the electron-poor benzopyranopyridine system). Again synthetic barriers were encountered, this time in synthesis of the parent ring system.

The synthetic pathway described by Kuo *et al.* (1989) could not be reproduced as outlined and closure of the ring system was not realised. Degradation to salicylic acid was the dominant reaction course and the usefulness of this system as a lead was in serious doubt. Considering these failures, we then returned to the original parent systems (benzopyranopyridine and benzothiopyranopyridine) and modified the desired side chain structures intended for the C-7 position. To this end, it was decided that we should pursue the azido functional groups as previous researchers in our laboratory determined that heterocyclic rings on the C-7 position afforded the best activity in the *in vitro* testing done on tracheal parenchyma strips. New heterocyclic rings at C-7 were not pursued as it was postulated that a ring may not be necessary for activity but rather a multi-heteroatom, electron-rich side chain would be all that was necessary for activity. These proved to be facile reactions which resulted in the compounds reported later in this thesis.

6.0 SYNTHESIS OF 5H-[1]BENZO(THIO)PYRANO[2,3-B]PYRIDINE PARENT RING SYSTEMS

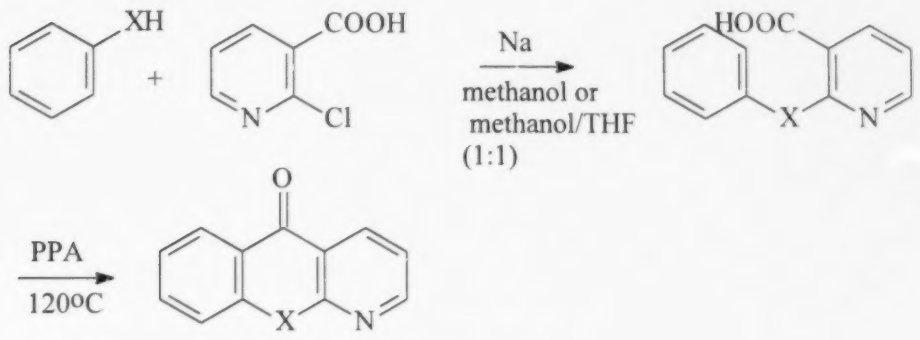
6.1 Benzo(thio)pyrano[2,3-b]pyridines:



5H-[1]benzopyrano[2,3-b]pyridine-5-one (X=O); 5H-[1]benzothiopyrano[2,3-b]pyridine-5-one (X=S).

Syntheses of the benzopyranopyridine and benzothiopyranopyridine parent ring systems were achieved following the method reported by Villani *et al.* (1975). Modifications were made on the parent systems at the C-7 position by nitration with KNO_3 and H_2SO_4, followed by reduction of the nitro group to an amine using two standard reaction conditions. Reduction of the amine was first attempted utilising Fe^{+++} and ammonia as was previously reported from our laboratory; however, yields were poor, the reaction was not consistent, and caused grief in cleaning the glassware. It was decided to pursue a more standard reduction method employing $SnCl_2$ and HCl/methanol (1:1). This method gave excellent (85-90%), reproducible yields and was the main reaction sequence utilised in production of both oxygen-containing and sulphur-containing parent systems (see Scheme I). The solvent employed in the synthesis of benzopyrano[2,3-b]pyridine was methanol, whereas the solvent in

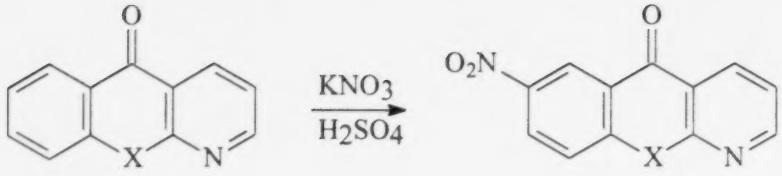
production of the benzothiopyrano[2,3-b]pyridine was a one to one mixture of tetrahydrofuran and methanol. Utilising pure methanol in the synthesis of the sulphur-containing parent ring system gave poor yields.



Scheme I: Production of benzopyranopyridine and benzothiopyranopyridine parent ring systems. X=S or O.

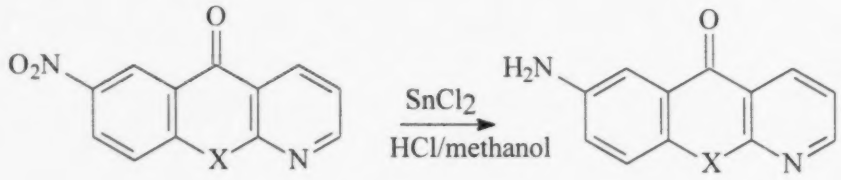
6.2 Ami[illegible]on of benzopyranopyridine and benzothiopyranopyridine:

Each parent system (benzopyranopyridine and benzothiopyranopyridine) was reacted with KNO_3 in concentrated H_2SO_4 in an ice bath for three hours (see Scheme II). At this time, the reaction was stopped by the addition of cold water which produced a thick, pale yellow paste which was then dried and the precipitate recrystallized from large volumes of acetonitrile. The resultant fluffy, white crystals were then used for the next step.



Scheme II: Production of 7-nitro compounds. X=O or S.

The 7-nitrobenzopyranopyridine and 7-nitrobenzothiopyranopyridine were then each reacted with $SnCl_2$ in order to reduce the nitro group to a primary amine (see Scheme III). The nitro compound was refluxed in HCl/methanol (1:1) in the presence of $SnCl_2$ crystals for two hours. At this time, the reaction was allowed to cool to room temperature and was basified with 40%^{w}/v NaOH. The resultant bright yellow paste was filtered, dried, and refluxed in acetonitrile to remove the excess $SnCl_2$ powder. When it was apparent that only a pale grey powder remained undissolved in the reaction flask, the solution was removed from heat and the grey powder filtered off. The resulting mother liquor was allowed to cool and solvent removed to reveal soft, fluffy, bright yellow crystals. Yields were consistently very high at 90-95%.

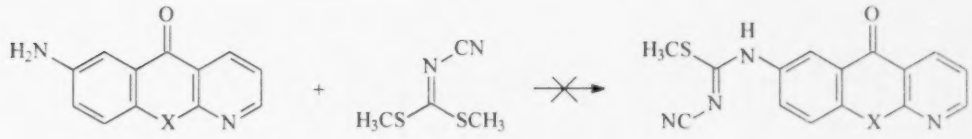


Scheme III: Production of 7-amino compounds. X=O or S.

7.0 MODIFICATION OF 7-AMINO-BENZO(THIO)PYRANOPYRIDINE:

7.1 Attempted addition of dimethyl N-cyanodithioiminocarbonate:

A wide variety of reaction conditions were evaluated for conversion of the C-7 primary amine into the desired guanidinyl and nitroethyl side chains (Scheme IV). Initially work by Hoffman *et al.* (1983) in their synthesis of cimetidine and that of Yamada *et al.* (1983) in the synthesis of antisecretory and antiulcer cyanoguanidine derivatives were considered and applied to the system at hand; however, despite numerous attempts and variation of reaction conditions, the desired reaction products were not evident.



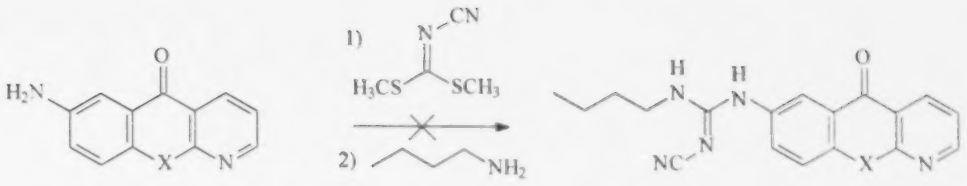
Scheme IV: Reaction of benzo(thio)pyranopyridine with dimethyl N-cyanodithioiminocarbonate. X=O or S.

Initially, reactions in ethanol at room temperature and at reflux were used (Hoffman, *et al.*, 1983), but these proved to be ineffective and more rigorous conditions were explored. Reaction temperatures were gradually increased from room temperature to 60°C and to reflux with no reaction evident.

An attempt to use microwave-promoted reaction (Abramovitch, 1991; Molina *et al.*, 1993) proved to be dangerous (several explosions occurred) and not useful: reaction times of 30 seconds produced no reaction and reaction times of one minute produced explosions. Initially solvent repeatedly evaporated from the reaction vessel so further sealing efforts were made (encasing the silicon screw-top byl in parafilm) but this only served to change the time of explosion from one minute to 30 seconds.

The SN_2 displacement reaction is generally favoured when a polar aprotic solvent is employed; therefore, DMF was used as solvent but to no avail. Reaction temperatures employed in the DMF reactions again ranged from room temperature to 60°C and to reflux but desired reaction products were not obtained.

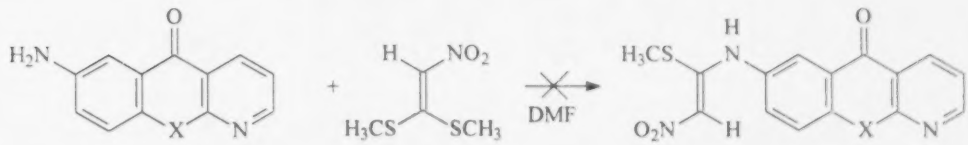
In order to see if the product was being formed, but degrading upon work-up, a one-pot synthesis was attempted utilising DMF as the solvent at varying times and temperatures, followed by the addition of *n*-butylamine (see Scheme V). The one-pot method was used in order to avoid the work-up step where it was proposed that the imidocarbamate was cleaving from the parent ring system, and so that the final guanidine product may have been realised. Again the desired product was not isolated. ^{1}H NMR was performed on the resultant yellow crystals but the product obtained did not produce expected ^{1}H NMR spectrum, nor were we able to rationalise the spectrum produced. It is unclear from these experiments if the imidocarbamate was adding to the parent benzopyranopyridine and degrading after the addition of the amine or if the imidocarbamate was indeed adding at all.



Scheme V: One-pot synthesis attempting to avoid potential cleavage of imidocarbamate from parent benzopyranopyridine during work-up.

7.2 Attempted addition of 1,1-bis(methylthio)-2-nitroethylene:

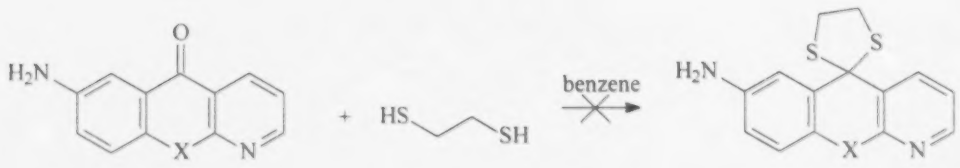
Addition of 1,1-bis(methylthio)-2-nitroethylene (see Scheme VI) employing the same variety of reaction conditions as were applied to the dimethyl N-cyanodithioiminocarbonate addition reactions, were also conducted but again desired product was not forthcoming. TLC (10% methanol in chloroform) repeatedly indicated the presence of only starting material in the reaction mixture.



Scheme VI: Reaction of benzo(thio)pyranopyridine with 1,1-bis(methylthio)-2-nitroethylene. X=O or S.

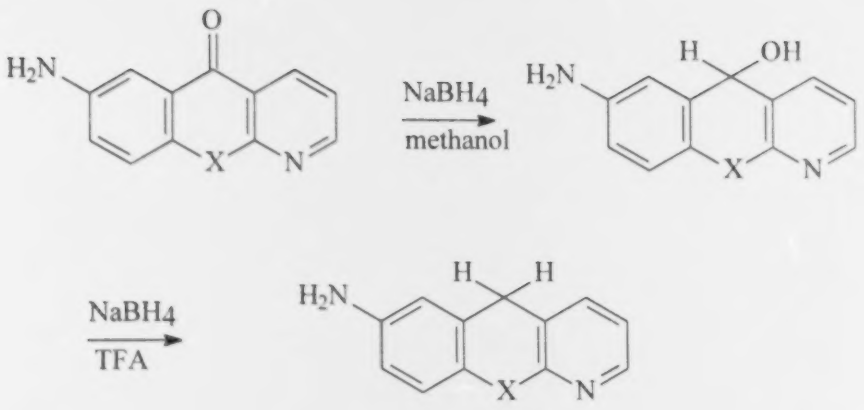
7.3 Modifications to C-5 carbonyl group:

It was proposed that the C-5 carbonyl group was reducing the reactivity of the C-7 amino group. Modifications to this carbonyl group were undertaken in order to determine if this was true. Initially, protecting the carbonyl group seemed a valid option. The parent ring system was reacted with ethanedithiol in BF_3·etherate (Hatch *et al.*, 1978) (see Scheme VII). After repeated attempts, protection was not realised. Utilising SiO_2·$SOCl_2$ (Kamitory *et al.*, 1986) as a catalyst was not helpful either, and it was decided that reduction of the carbonyl to a methylene was a better choice.



Scheme VII: Attempted protection of C-5 carbonyl with ethanedithiol. X=O or S.

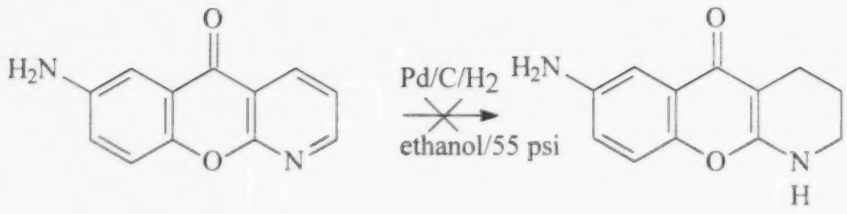
The conditions for reduction of the benzopyranopyridine (and benzothiopyranopyridine) C-5 ketone which were determined previously by researchers in this laboratory and elsewhere (Nutaitis *et al.*, 1991) were employed. Reduction was successful (see Scheme VIII); however, the C-5 methylene ring systems were still inactive toward reaction with the imidocarbamate and nitroethylene reactants.



Scheme VIII: Reduction of C-5 carbonyl to methylene. X=O or S.

7.4 Reduction of pyridine ring:

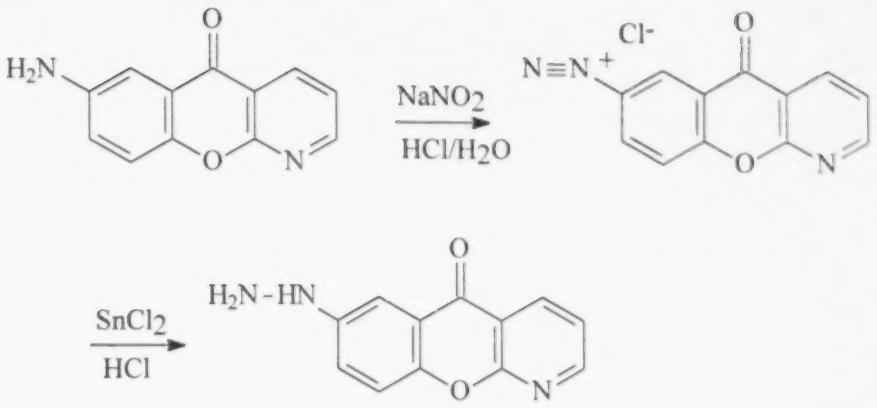
It was proposed that the pyridine ring was sufficiently electron withdrawing that it might be reducing the reactivity of the C-7 amino group. Reduction of the pyridine group was unsuccessful using Pd/H_2 on activated charcoal with ethanol as solvent (see Scheme IX). It is likely that the reaction failed due to an improperly functioning Parr Hydrogenater since this reaction was previously successful in our laboratory.



Scheme IX: Attempted reduction of pyridine group to the 1,2,3,4-tetrahydropyridine.

7.5 Formation of hydrazine group at C-7:

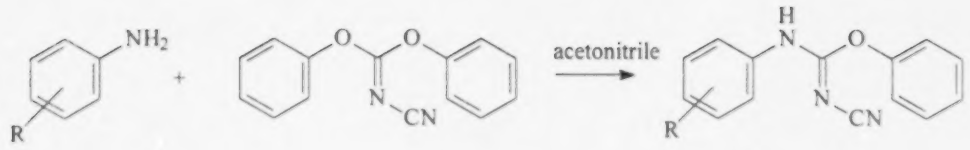
It was proposed that extension of the amino group away from the parent ring would decrease the effect of the parent on the reactivity of the amino group. The hydrazine was determined to be the most accessible chain extension and it was formed under conditions developed by previous researchers in the laboratory (Murthy, 1988). Production of the hydrazine was successful (see Scheme X); however, it was not effective in increasing the reactivity of the terminal amino group and addition of the desired side chains was not realised.



Scheme X: Production of hydrazine group with proposed increased reactivity.

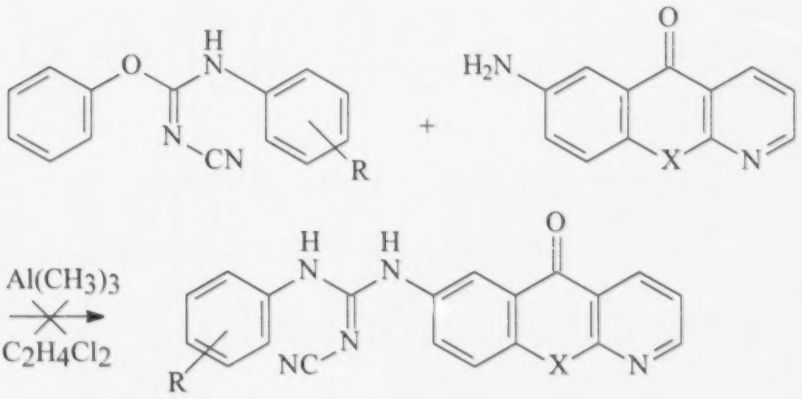
7.6 Modification of C-7 utilising trimethylaluminum as a catalyst:

Further attempts at modification on the C-7 position were made using the synthesis outlined by K.S. Atwal *et al.* (1994) in their synthesis of BMS-180 448. Desired amines (diethylamine, *p*-hydroxyaniline, *o*-hydroxyaniline, and *m*-hydroxyaniline) were reacted with diphenyl cyanocarbonimidate (Garratt *et al.*, 1989) using acetonitrile as the solvent (see Scheme XI and Table 1). The various amines produced were then reacted with the benzopyranopyridine parent system under a nitrogen atmosphere and worked-up (Atwal *et al.*, 1994) but product obtained did not produce expected ^{1}H NMR spectrum, nor were we able to rationalise the spectrum produced (see Scheme XII).



Scheme XI: Representative displacement reaction between amines and diphenyl cyanocarbonimidate. R=OH or H.

Reactions were performed with both the diphenyl cyanocarbonimidate and dimethyl N-cyanodithioiminocarbonate to produce the desired amines. Differences in reactivity were not observed between the two; however, the phenoxy group was thought to be a better leaving group than the methylthio group and the majority of amines were produced using this compound.



Scheme XII: Attempted coupling of benzopyranopyridine parent to various amino phenyl cyanocarbonimidates. X=O or S; R=OH or H.

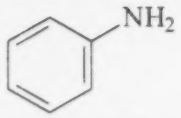
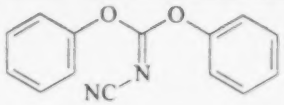
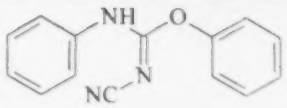
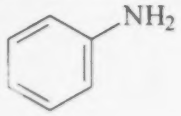
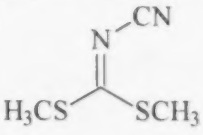
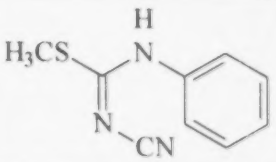
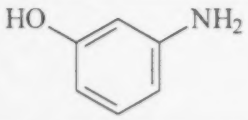
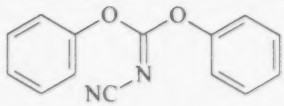
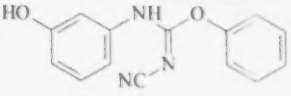
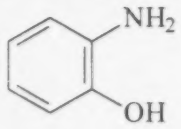
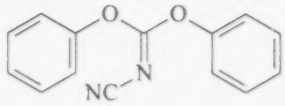
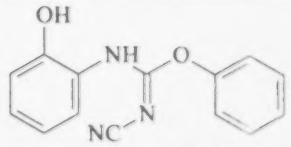
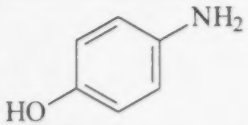
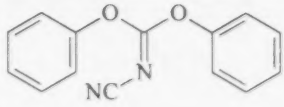
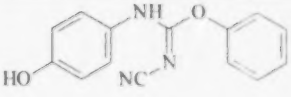
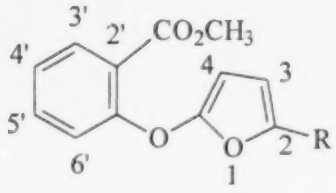
Amine Reacted	cyanocarbonimidate	Compound Produced
		
		
		
		
		

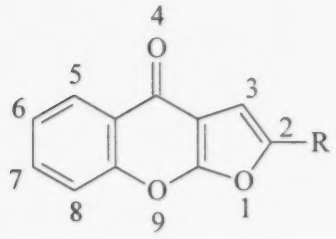
Table 1: Amines produced for reaction with benzo(thio)pyranopyridines.

At this point, the synthetic failures made us change our focus to the electron-rich furochromone ring system.

8.0 SYNTHESIS OF AND MODIFICATIONS TO FURO[2,3-B]CHROMONE LEAD RING STRUCTURE:



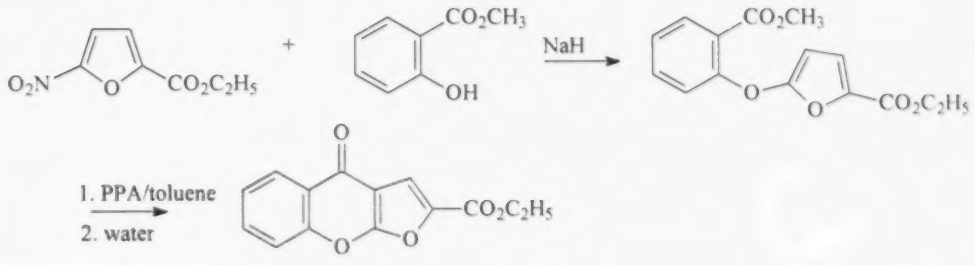
5-(2'-methoxycarbonylphenoxy)furan-2-derivatives



furo[2,3-b]chromone-2-derivatives

The furo[2,3-b]chromone parent system was considered for use as a lead compound because of its close relation to the psoralens (naturally occurring furocumarines isolated from various plant sources including bergamot and cloves), which have been recognised as effective for a number of years in treatment of inflammatory diseases such as psoriasis (a disease with known connections to AA cascade products, including the leukotrienes). The furo[2,3-b]chromones were also considered to be logical targets due to their similarity to the hydroxamic acid A-78 773 and its derivatives (Bell *et al.*, 1993). The paper by Kuo *et al.* (1989) was the

only one found in the literature search on this particular ring arrangement and it was used as the basis for synthesising the parent system. Unfortunately, it became apparent that the results reported in that paper were not reproducible. The reaction conditions reported by Kuo *et al.* did not result in the production of the desired product nor did it make logical sense in some of the reactions it described. An example was the assertion that purification of the parent furochromone on a silica column produced the C-2 ethyl ester from the C-2 carboxylic acid. The reaction reported by Kuo *et al.* is outlined in Scheme XIII below.



Scheme XIII: Reaction outlined in Kuo *et al.* 1989.

9.0 ALTERATIONS TO FURAN STARTING MATERIALS:

9.1 Modification at C-2 on 5-nitro-2-furoic acid:

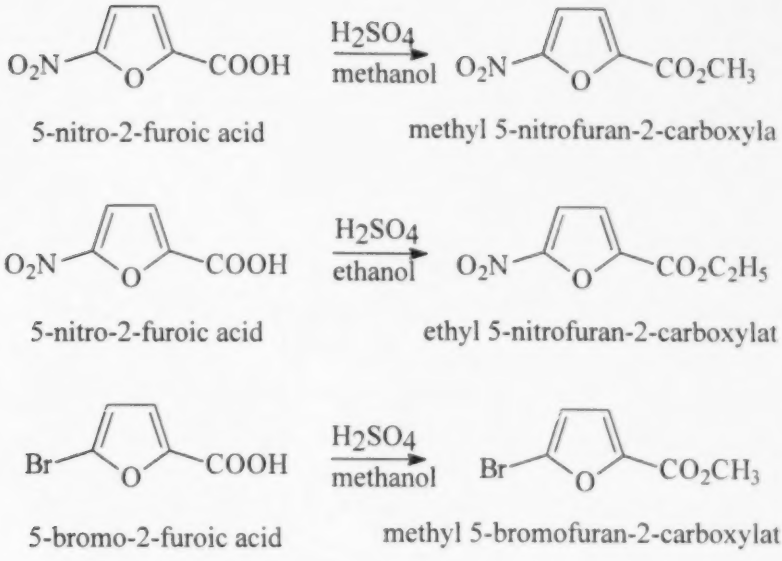
A variety of alterations were made to the furan starting materials in order to increase the likelihood of the desired nucleophilic attack by the ionised hydroxy group of methyl salicylate with which it was to be reacted (see Scheme XIV for

reaction performed). It was determined that the aldehyde group in the C-2 position of the furans afforded the best reactivity to nucleophilic attack (coupling between methyl salicylate and 5-nitro-2-furaldehyde gave reaction yields approaching 80%); however, because early experimentation with ring closure to the tricyclic system gave no desired compound, it was assumed that the ester, such as the ethyl ester used in Kuo *et al.*, may be necessary for the reaction to proceed.

Because the aldehyde was quite reactive, but assumed to be undesirable, the ethyl ester on the C-2 position of the 5-nitro-2-furoic acid was synthesised because it was reported by Kuo *et al.*, (1989) to be the appropriate starting furan. The methyl ester on C-2 of the 5-nitro-2-furoic acid was also synthesised. The reaction producing the methyl ester was facile and gave good yields (80-90%). Various other side groups at C-2 of the 5-nitro-2-furoic acid were also synthesised (Scheme XIV) or purchased (Figure 1), including the carboxylic acid and nitrofurantoin, in order to test their reactivity and to possibly reduce the need for further modification at C-2 after cyclization was realised.

9.2 Exploration of most reactive leaving group on C-5 of various furans:

The NO_2 group was considered to be the best leaving group, because when the NO_2 was replaced with a Br group, no appreciable reaction between methyl salicylate and the furan was noted. The 5-bromofuroic acid was reactive to esterification (Scheme XIV) but not to coupling with methyl salicylate. Also, the 5-bromo-2-furaldehyde (Figure 1) was not reactive in the coupling with methyl salicylate. Both reactions failed to give desired products.



Scheme XIV: Variations on 2,5-furanoic starting materials.

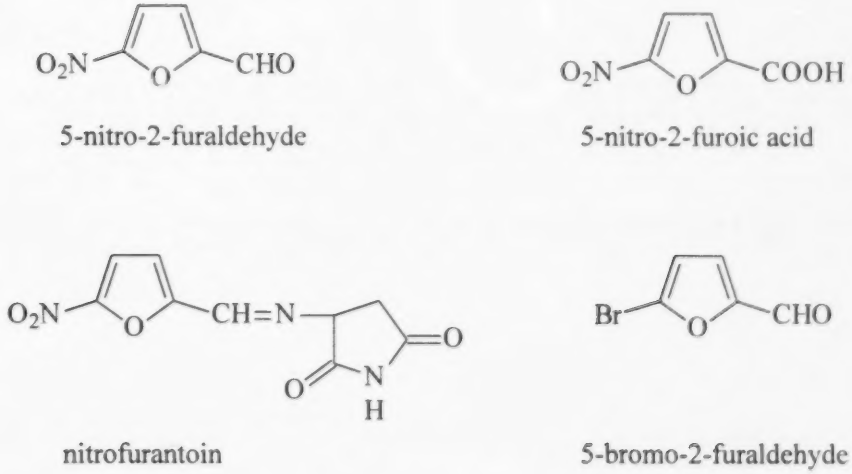
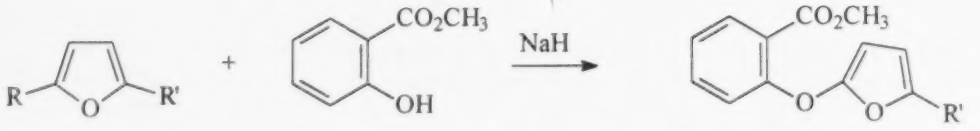


Figure 1: Other furans used as starting materials.

9.3 Reaction between furan starting material and methyl salicylate:

The coupling of methyl salicylate to the various furan starting materials (Scheme XV) was generally quite good (yields upwards of 60% for the methyl 5-nitrofuran-2-carboxylate). Although the aldehyde functional group at C-2 (R'=CHO) gave very good yields (up to 80%) in this stage of the reaction, it was supposed that it deactivated the 5-(2'-methoxycarbonylphenoxy)furan toward cyclization as desired product could not be recovered after cyclization reactions.

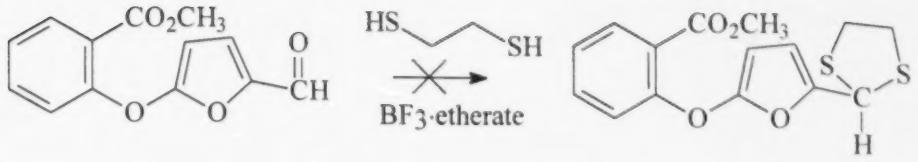


5-(2'-methoxycarbonylphenoxy)furan

Scheme XV: Reaction to produce 2-substituted 5-(2'-methoxycarbonylphenoxy)furans. R=NO_2 or Br. R'=COOH, CHO, CO_2CH_3, or $CO_2C_2H_5$

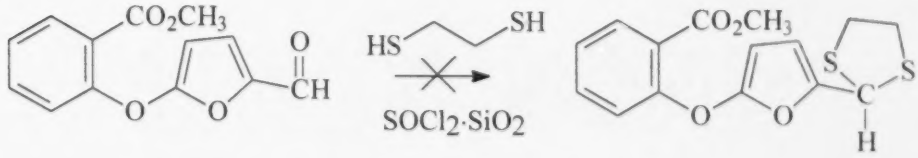
9.4 Attempted protection of C-2 aldehyde of 5-nitro-2-furaldehyde:

It was proposed that the aldehyde group present on the 5-nitro-2-furaldehyde was sufficiently electron withdrawing to prevent cyclization of the methyl salicylate and the furan (the final step in production of the desired furochromone). Protection of the C-2 aldehyde group was proposed as a means to reduce the electron withdrawing effects of the aldehyde group. Protection with ethanedithiol was attempted utilising BF_3·etherate as catalyst (Hatch *et al.*, 1978) but desired product was not recovered (Scheme XVI).



Scheme XVI: Attempted protection of 5-(2'-methoxycarbonylphenoxy)furan-2-aldehyde.

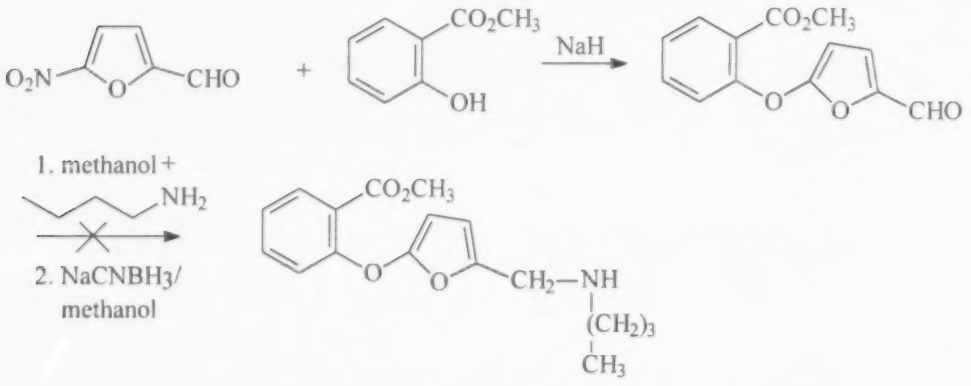
More recent literature sources suggest protection of aromatic aldehydes can be realised utilising silica gel treated with thionyl chloride as a catalyst (Scheme XVII) (Kamitori, *et al.*, 1986). The silica catalyst was prepared by addition of thionyl chloride to silica suspended in CH_2Cl_2. The fresh catalyst was utilised in the protection reaction but product obtained did not produce expected ^1H NMR spectrum, nor were we able to rationalise the spectrum produced. Further attempts at protection of the aldehyde were thus abandoned.



Scheme XVII: Protection of 5-(2'-methoxycarbonylphenoxy)furan-2-aldehyde.

9.5 Attempted reaction of 5-(2'-methoxycarbonylphenoxy)furan-2-aldehyde with *n*-butylamine:

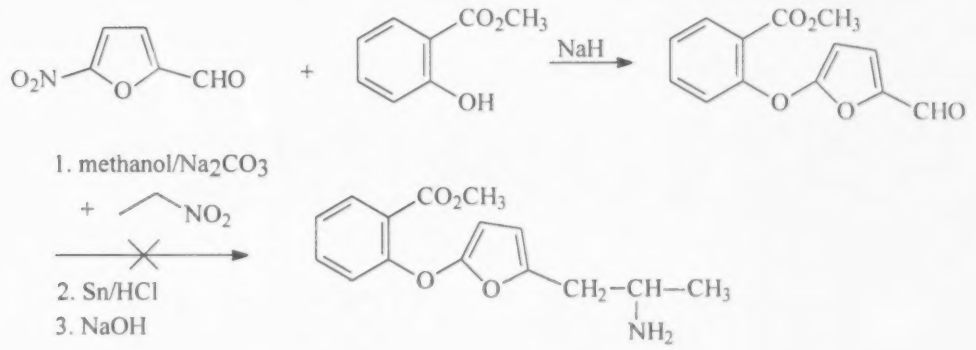
Modification of 5-(2'-methoxycarbonylphenoxy)furan-2-aldehyde prior to cyclization was attempted in order to reduce the electron withdrawing nature of the aldehyde group as well as to remove the need for modification after cyclization. A straight chain aliphatic amine, *n*-butylamine, was chosen for the reaction (see Scheme XVIII). The *n*-butylamine was refluxed with 5-(2'-methoxycarbonylphenoxy)furan-2-aldehyde followed by reduction of the imino group with $NaCNBH_3$. The reaction was unsuccessful as TLC (10% methanol in chloroform) revealed a multitude of components. ^1H NMR was performed on product recovered from preparative TLC which indicated decomposition of the ring system.



Scheme XVIII: Attempted addition of *n*-butylamine to 5-(2'-methoxycarbonylphenoxy)furan-2-aldehyde.

9.6 Attempted reaction of 5-(2'-methoxycarbonylphenoxy)furan-2-aldehyde with nitroethane:

Modification of the aldehyde by reaction with nitroethane (Pearl and Beyer, 1951) was also attempted. It was again postulated that reaction with nitroethane to give a terminal amine would be appropriate functionality for the 5-(2'-methoxycarbonylphenoxy)furan as further modification at the terminal amine produced would be possible. The nitroethane was stirred together with the 5-(2'-methoxycarbonylphenoxy)furan-2-aldehyde to be followed by reduction of the nitro olefin groups. Addition was not realised (see Scheme XIX) as TLC (chloroform) indicated the presence of a multitude of components which could not be identified.

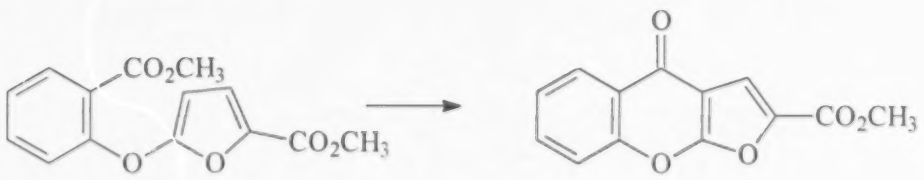


Scheme XIX Attempted addition of nitroethane to 5-(2'-methoxycarbonylphenoxy)furan-2-aldehyde.

9.7 Choice of methyl 5-nitrofuran-2-carboxylate for future reactions:

At this point a decision to proceed with one furan derivative was made. The methyl ester was chosen to be the best furan with which to continue as it was easy to produce in excellent yields as mentioned above. It also gave good yields in the coupling reaction with methyl salicylate. Also as noted above, the NO_2 group at C-5 was the best leaving group as the Br-substituted furans gave no product when reacted with methyl salicylate and the nitro-substituted furans gave yields upwards of 80%. Based on these features, the methyl 5-nitrofuran-2-carboxylate derivative of furan was utilised in the majority of attempts at cyclization to the desired furo[2,3-b]chromone ring system.

10.0 ATTEMTPED CYCLIZATION OF FUROCHROMONE RING SYSTEM:



Scheme XX: Proposed cyclization to furo[2,3-b]chromone.

Kuo *et al.*, (1989) describe the cyclization reaction to be a standard ring closure using polyphosphoric acid (PPA) and toluene, but then it was followed by stirring in water at room temperature for 18 hours. The method described was followed exactly but could not be reproduced (see Scheme XX). Modifications to the

method included: decreasing the time and temperature of the reaction; removing the toluene and water from the process; utilising alternative ring closure methods such as $AlCl_3$ in CS_2 or in nitrobenzene. None of the methods was successful: ring cleavage of the furan and cleavage of the furan from the salicylate were always the predominating reaction outcomes.

10.1 Attempted duplication of cyclization conditions reported by Kuo *et al.*, (1989):

The methyl 5-(2'-methoxycarbonylphenoxy)furan-2-carboxylate was dissolved in benzene and stirred in PPA for 6 hours at 100°C then cooled and diluted with ice water and further stirred overnight. The reaction was worked-up according to Kuo *et al.* (1989) but only salicylic acid was recovered. Despite repeated attempts utilising this method salicylic acid was consistently obtained.

10.2 Reaction variations utilising PPA as cyclizing agent:

Cyclization of 5-(2'-methoxycarbonylphenoxy)furan-2-carboxylic acid to the furo[2,3-b]chromone utilising PPA in the same manner as was successfully applied to cyclization of the benzopyrano[2,3-b]pyridines was attempted. Reaction temperatures of 80°C, 100°C, 120°C and 180°C were used with similar results: reaction temperatures above 100°C consistently gave unidentifiable tars, and reactions at 80°C rapidly produced salicylic acid.

The time at which decomposition to salicylic acid was occurring was determined to be approximately between 15 and 30 minutes. The TLC (chloroform) at 15 minutes indicated reaction had not occurred yet the TLC at 35 minutes indicated decomposition to unidentifiable tar (see Table 2). The reaction utilising PPA as a cyclizing agent was abandoned because of this experiment.

Reaction conditions:

Attempt #	wt. of PPA (1+2)	wt of furanoic acid	"phase 1" (minutes)	"phase 2" (minutes)	"phase 3" (minutes)
#1	2g + 1g	250 mg	~3	~4	~5
#2	1.5g + 1g	150 mg	10	4.5	20
#3	1.3g +0.9g	150 mg	5	5	10

Procedure:

The PPA was warmed on a steam bath to approximately 80°C to which the 5-(2'-carboxyphenoxy)furan-2-carboxylic acid was added. The mixture was stirred vigorously with a glass rod for the specified time in "phase one". The mixture was returned to the steam bath and the second portion of PPA was added. "Phase two" saw the reaction again vigorously stirred on the steam bath for the specified time. The flask was then removed from the bath and allowed to cool for the time specified in "phase three" after which ice water was added and product extracted with chloroform.

Results:

Attempt #1	a brown oil produced; TLC (chloroform) indicated two spots.
Attempt #2	an off-white oil produced; TLC indicated a multitude of spots; two chosen for preparative TLC but no product isolated
Attempt # 3	TLC again showed several spots, both in the chloroform extract and the precipitate in the acid layer

Table 2: Summary of experiments to determine time to decomposition.

10.3 Conversion of 5-(2'-carbonylphenoxy)furan-2-carboxylic acid to the acid chloride:

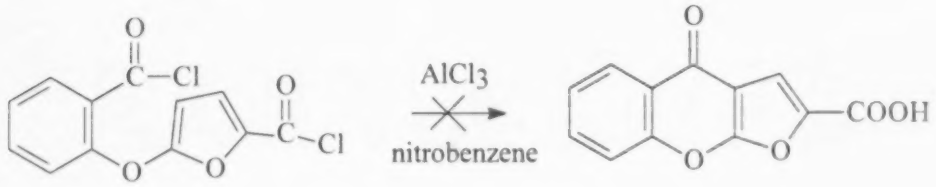
The acid chloride derivative of 5-(2'-carbonylphenoxy)furan-2-carboxylic acid was prepared by suspending the furanoic acid in chloroform and carefully adding thionyl chloride and warming the mixture to 80°C for 45 minutes (see Scheme XXI). It was unclear if the acid chloride was being produced; low resolution ^{1}H NMR (90 MHz) indicated the acid chloride was likely present, but melting points of the acid chloride and the furanoic acid were very similar (165°C and 167°C, respectively).

Scheme XXI: Production of acid chloride of 5-(2'-carbonylphenoxy)furan-2-carboxylic acid.

10.4 Attempted cyclization of 5-(2'-carbonylphenoxy)furan-2-carboxylic acid chloride to furo[2,3-b]chromone:

The 5-(2'-carboxyphenoxy)furan-2-carboxylic acid chloride and $AlCl_3$ were stirred together in nitrobenzene (which had been purified and dried) at 100°C. An accurate TLC (10% methanol in chloroform) was difficult to obtain as the nitrobenzene obscured and altered the spot migration. To get around this problem, small portions of the reaction mixture were removed, hydrolysed with 40%^{w}/v NaOH

and acidified with concentrated HCl in order to see the compound without the nitrobenzene interfering. The reaction was allowed to proceed overnight at which point TLC indicated a reaction had occurred (see Scheme XXII). TLC did not give a good indication of the stage of the reaction because the spots were smeared from baseline to the final spot (despite removal of the nitrobenzene). At this point the reaction was cooled and worked up as for the TLC preparation because of the total distance of spot (smear) migration. The water was removed *in vacuo* by roto-vap leaving a large quantity of NaCl and potentially the desired product. The dry crystals were washed well with chloroform, solvent collected and then removed *in vacuo* by roto-vap to leave a small amount of beige precipitate which was recrystallized from ethanol-water.



Scheme XXII: Attempted cyclization of 5-(2'-carboxyphenoxy)furan-2-carboxylic acid chloride to furo[2,3-b]chromone.

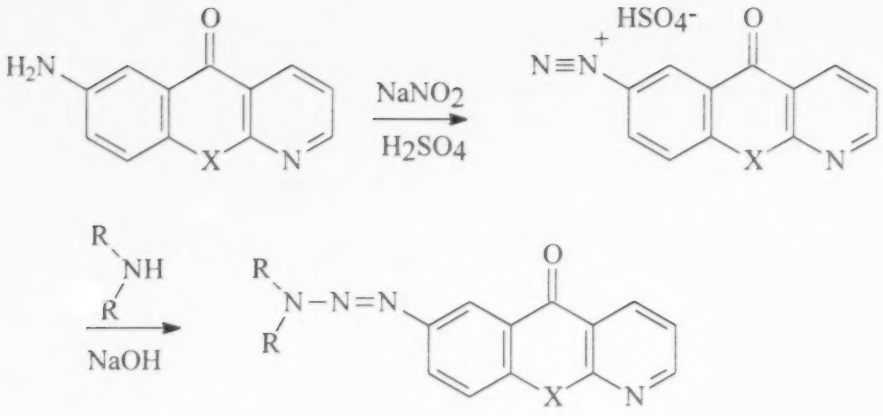
Initial ^{1}H NMR studies of the proposed furo[2,3-b]chromone were encouraging, however, ^{13}C NMR and EI-MS (low and high resolution), and CI-MS were conflicting and pointed to the identity being salicylic acid. ^{1}H NMR de-coupling experiments were indicative of a single aromatic ring, likely salicylic acid. ^{13}C NMR

(deuterated chloroform solvent) showed six carbons were present and high resolution mass spectrometry indicated a molecular ion of 138.032 amu ($C_7H_6O_3$) whereas a molecular ion of 202 amu was predicted. The base peak of m/z=120.021 amu ($C_7H_4O_2$) could not be explained if the desired product was indeed present. Low resolution CI (NH_3 ionisation gas) indicated a molecular ion of 273 amu which was not expected and remains unexplained. No peaks were present at proposed M+18 of 220 amu which would have been seen if the desired compound was present. The same procedure was followed using carbon disulphide as the solvent, but results were similarly discouraging.

After experimentation with these various reaction conditions, this ring system was abandoned and focus was returned to the benzopyranopyridine (and benzothiopyranopyridine) system and to the azido side chains on C-7.

11.0 PREPARATION OF 7-DIAZAMINO BENZO(THIO)PYRANO[2,3-B]PYRIDINE COMPOUNDS:

C-7 Substituted benzopyranopyridine and benzothiopyranopyridine series of compounds ranging from short chain di-amino groups, to branched alkyl, to di-aromatic and finally cyclic amines were synthesised and identified. Secondary amines were necessary as the reaction with primary amines was unpredictable and resulted in a variety of products. Two primary amines, propylamine and phenethylamine were used, but the products recovered could not be sufficiently purified to give clear indication of their identities.



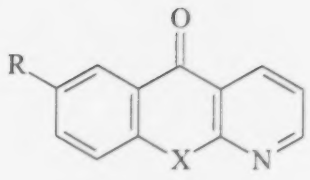
Scheme XXIII: Reaction for addition of secondary amino groups to the C-7 position of the benzo(thio)pyrano[2,3-b]pyridine compounds. X=O or S. R=methyl, ethyl, *n*-propyl, isopropyl, phenyl, benzyl, morpholino, pyrrolidino, and N-benzylpiperazino.

All derivatives in the two series were produced in a similar manner (see Scheme XXIII). Initially the 7-aminobenzopyranopyridine (or 7-aminobenzothiopyranopyridine) was reacted with $NaNO_2$ in 50%^{v}/v H_2SO_4 at 0-5°C for two hours to produce the 7-diaza derivative utilising a modification of the procedure used by Lin and Kasina (1981) in the modification of phenothiazines. With continued stirring in the ice bath, either eight or four molar equivalents of the desired secondary amine were added and stirred for a further half hour (most of the benzopyranopyridine derivatives were produced with eight molar equivalents; whereas, most of the benzothiopyranopyridine derivatives were synthesised utilising four molar equivalents). At this point the reaction was basified with 40%^{w}/v NaOH

and one of two work-up procedures was followed. The benzopyranopyridine derivatives were immediately removed from the stir-plate and the resultant precipitate filtered through a sintered glass funnel. The precipitate was washed repeatedly with ethyl acetate as was the filtrate. The washings were combined, dried with $MgSO_4$, and solvent removed *in vacuo* to reveal the product which was then further purified by crystallisation from acetonitrile.

A better procedure for work-up was devised when the majority of the benzothiopyranopyridines were being synthesised and was used from that point on. The reaction work-up was altered after basification. Instead of removing the reaction from the stir-plate, 100 mL water and 25 mL chloroform were added and the reaction was stirred at room temperature for one hour. This step afforded better separation of product from the salt formed when the NaOH was added. The mixture was separated by separatory funnel, which saw the aqueous layer washed with chloroform (4 by 25 mL portions), dried with $MgSO_4$, and solvent removed *in vacuo* by roto-vap. The resultant product also required crystallisation from acetonitrile, but the work-up procedure was cleaner and afforded better separation.

Addition of the desired amines was clean and proceeded with good yields for all side chains except for the diphenyl and dibenzylamines (see Table 3). These groups were probably too bulky which may have prevented the addition of the amine to the diaza function on the parent molecules. The large aromatic groups of the diphenylamine and dibenzylamine may have also reduced the reactivity of the amine group, thus preventing it from attacking the diaza function on the benzopyranopyridine (and benzothiopyranopyridine).



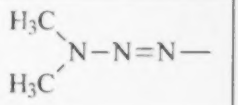
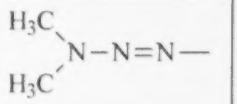
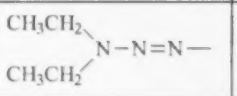
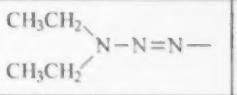
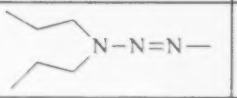
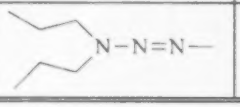
R	X	melting point	yield %
	O	199-201°C	75%
	S	169-171°C°	19%
	O	131-132°C	80%
	S	101-103°C	35%
	O	92-94°C	50%
	S	70°C	12%

Table 3: 7-Diazaminobenzo(thio)pyrano[2,3-b]pyridines produced.

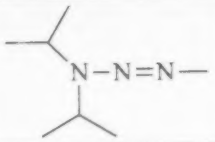
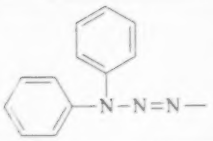
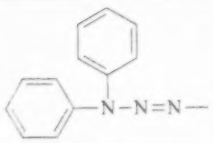
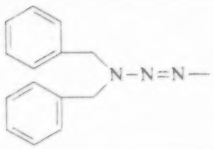
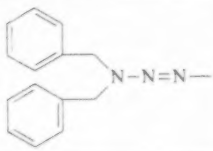
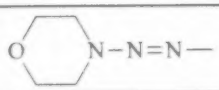
	S	182°C	15%
	O	oil	product not isolated
	S	oil	product not isolated
	O	oil	product not isolated
	S	197°C	^{1}H NMR, IR, and MS data do not support proposed structure.
	O	170°C	13%

Table 3 (continued): 7-Diazaminobenzo(thio)pyrano[2,3-b]pyridines produced.

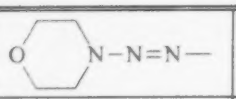
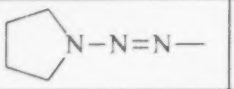
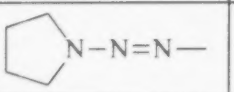
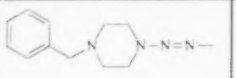
	S	205-207°C	25%
	O	165°C	68%
	S	172-173°C	57%
	O	135°C	^{1}H NMR and MS data do not support proposed structure.

Table 3 (continued): 7-Diazaminobenzo(thio)pyrano[2,3-b]pyridines produced.

11.1 Common features in the physical data:

All of the compounds produced were expected to produce similar patterns as only the side chains differed between the compounds. ^{1}H NMR data were expected to give: a doublet of doublets at approximately 8.8δ representing H-2; a doublet of doublets at 8.7δ representing H-4; a doublet at 8.5δ representing H-6; a doublet of doublets at 7.8δ representing H-8; a doublet at 7.6δ representing H-9 and a doublet of doublets at 7.4 δ representing H-3. The side chains which were successfully added were all aliphatic in nature and appeared further upfield in the aliphatic range. Proton H-6 appears somewhat further downfield than expected due to the effects of the

adjacent C-5 carbonyl and also due to the electron withdrawing nature of the diazamine side chains on C-7.

The IR spectral data are mostly unremarkable as the carbonyl peak at 1669 cm^{-1} was the main diagnostic feature. The absence of this peak was found in the products of the reactions which attempted addition of the aromatic amines to the benzo(thio)pyranopyridine parent. This feature allowed the immediate recognition that the amines had not in fact undergone the desired addition. The peak at 1669 cm^{-1} is where one would expect to see a carbonyl in a large aromatic system such as those at hand.

The EI-MS data were again very similar for all compounds produced. The compounds all gave molecular ion peaks and then proceeded to fragment in a similar manner (see Figures 2 and 3). Initially, the side chain is lost with the charge localised on the nitrogen attached directly to the parent ring system, then a molecule of nitrogen (N_2) is lost to give the base peak.

The benzopyranopyridine derivatives all gave a base peak of m/z=196 amu. Following the formation of the base peak ion, the parent ring fragments by the loss of carbon monoxide to produce ion m/z=168 amu, and then further fragments with the loss of another molecule of carbon monoxide to give m/z=140 amu. The ring system

also fragments with the loss of HCN from the base peak ion to give fragment ion m/z=169 amu.

The benzothiopyranopyridine derivatives give a base peak of m/z=212 amu. The base peak ion then either loses a molecule of carbon monoxide to give fragment ion m/z=184 amu, or by the loss of C_2H_2N to give ion m/z=172 amu. This ion of 172 amu then fragments with a loss of a sulphur atom to give ion m/z=140 amu ($C_{10}H_4O$). These fragmentation patterns are consistent for all compounds produced.

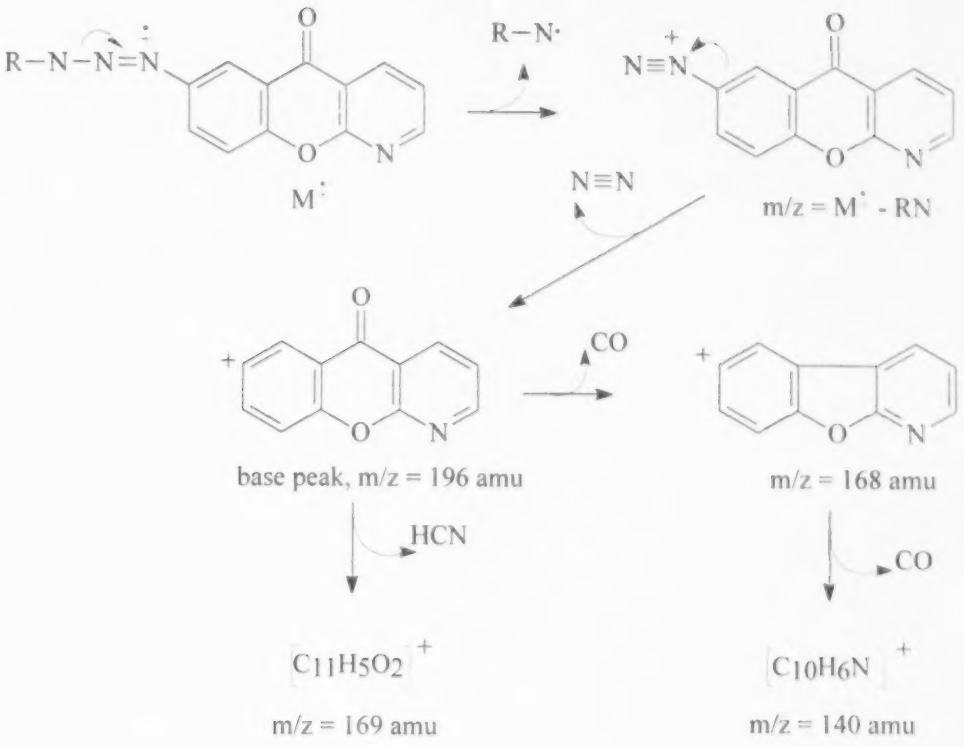


Figure 2: Proposed fragmentation of 7-diazamino benzopyranopyridine derivatives.

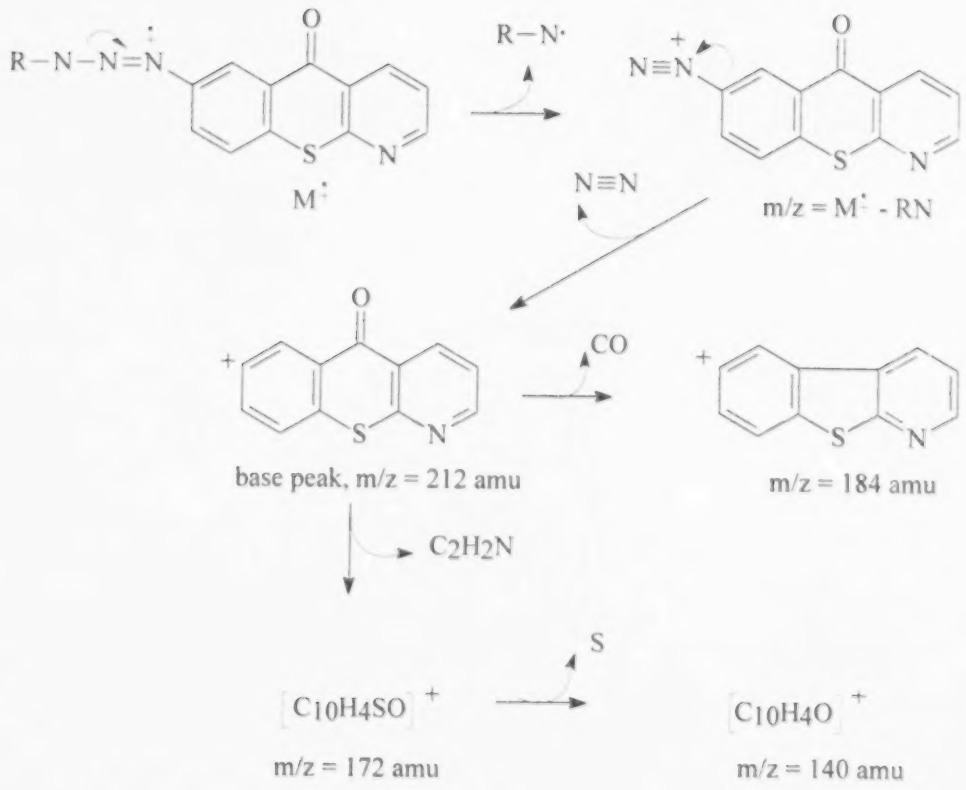


Figure 3: Proposed fragmentation pathway of 7-diazamino benzothiopyranopyridine compounds.

CONCLUSIONS:

The benzopyranopyridines and benzothiopyranopyridines remain an interesting group of compounds. Although at this point in time their mechanism of action is unclear, the potential for them to be acting as potassium channel agonists is an exciting prospect.

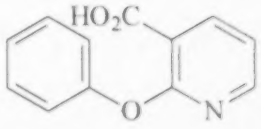
Throughout this project numerous synthetic barriers were encountered. Initial and subsequent attempts to add the guanidinyl and nitroethyl groups to the C-7 position of both benzopyrano[2,3-b]pyridine and benzothiopyrano[2,3-b]pyridine remain unrealised. Despite the various approaches taken, addition was not forthcoming. Attempts using ethanol as a reaction solvent were unsuccessful. Reaction at room temperature, 60°C, and reflux and at a wide variety of reaction times lead to recovery of only starting material. Utilising microwave promotion of the reaction lead to unidentifiable tar production. When the solvent was changed to dimethylformamide, again only starting material was recovered. Trimethylaluminum promotion of the reaction was also unproductive. Despite the fact that a reaction did occur, it was not the one desired as products were unidentifiable.

Synthesis of the furo[2,3-b]chromone ring system also remains unfulfilled. Again despite numerous attempts at a large variety of synthetic procedures, no cyclization to this three-ring parent was realised. Cyclization utilising polyphosphoric acid or $AlCl_3$ was not forthcoming and only fragmentation of the furan ring and degradation to salicylic acid was seen.

Synthesis of the 7-diazamino benzo(thio)pyrano[2,3-b]pyridine compounds was successful and utilised a one-pot method with short reaction times and relatively easy work-up. Attachment of amines to the hydrazine formed at C-7 of the benzo(thio)pyrano[2,3-b]pyridines was facile and repeatedly gave acceptable yields (12 to 80%). The compounds produced await biological testing at a future date.

EXPERIMENTAL:

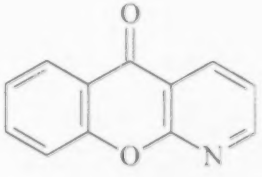
Melting points were all performed using the Mel-Temp II and are uncorrected. NMR spectra (^{1}H and ^{13}C) were performed using the Brunker AM 300 Fourier Transform spectrometer with or without an internal standard of TMS. Mass Spectra were recorded using the Trio 2000 spectrometer available at the Faculty of Pharmacy (low resolution) or using facilities in the Department of Chemistry (AEI-MS50, high resolution and AEI-MS12, chemical ionisation with NH_3 ionisation gas). IR spectra were obtained with either Nicolet 5DX or Nicolet Macro 550 spectrometers. Column chromatography utilised Terochem 100-200 mesh silica gel (pH 7.3, equivalent to Merck 7734) and solvents utilised for elution are mentioned within the body of the experiment. All TLC, other than preparative TLC, was done on approximately 5 μl. of cooled reaction mixture on aluminum-backed, commercially available silica TLC plates. Identification of previously reported compounds was considered confirmed if the ^{1}H NMR and melting points gained from compounds produced matched those previously reported from our laboratory (Murthy, 1988; Vudathala, 1990) or the original synthesis by Villani *et al.* (1975).



12.1 Preparation of 2-phenoxynicotinicacid:

Na metal (3 g, 130.4 mmol) was shaved into warming methanol (100 mL) and allowed to fully react for half an hour at 100°C at which point 20 g (212.8 mmol) of phenol was added. The reaction was stirred under reflux for a further half hour, at which time 5 g (31.7 mmol) 2-chloronicotinic acid was added and the mixture was

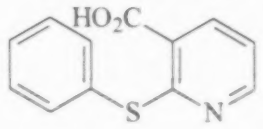
stirred for another half hour. At this time the temperature was raised to 160°C, the solvent was removed by distillation, and the melt was heated for two hours. The reaction was cooled and diluted with 50 mL water. The mixture was washed with ether (4 by 20 mL portions), acidified with conc. HCl, cooled, and the precipitate collected and dried. The resultant product was recrystallized from acetonitrile and yielded 1.56 g (26.0 mmol) cloth-like beige crystals with a melting point of 182°C. ^{1}H NMR ($CDCl_3$): 13.0δ (broad s, COOH); 8.25δ (m, overlapping H-2 and H-4, J=8, 4, 6, and 7 Hz); 7.42δ (t, H-7 and H-9, J=8 Hz); 7.24δ (m, overlapping H-8 and H-3, J=6, 7, and 8 Hz); 7.10δ (m, overlapping H-6 and H-10, J=7 and 8 Hz).



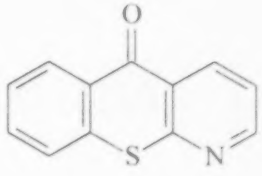
12.2 Preparation of benzopyrano[2,3-b]pyridine:

2-Phenoxynicotinic acid (3.4 g, 15.8 mmol) was stirred with a mechanical stirrer into 200 g PPA, warmed to 100°C, and allowed to react for 20 hours. The reaction was cooled, diluted with 400 mL cold water in an ice bath and allowed to cool to produce a thick precipitate. The precipitate was filtered, dried, and recrystallized from 95% ethanol to yield 2.8 g of solid (14.2 mmol) with a sharp melting point of 182°C (Villani, 178-182°C). ^{1}H NMR ($CDCl_3$): 8.74δ (dd, H-2, J=4 and 2 Hz); 8.75δ (dd, H-6, J=7 and 2 Hz); 8.33δ (dd, H-4, J=8 and 2 Hz); 7.79δ (m, H-8, J=9 and 2 Hz); 7.64δ (d, H-9, J=8 Hz); 7.46δ, 7.45δ (q, overlapped with dd, H-3 and H-7, J=5 and 2 Hz (H-3), J=7 and 1 Hz (H-7)).

12.3 Preparation of 2,3'-carboxypyridyl phenyl sulphide:



Na metal (3 g, 130.4 mmol) was shaved into 100 mL warming methanol/THF (1:1) and allowed to react fully for half an hour at 100°C; to this was added 30 mL (293 mmol) of thiophenol. Stirring was continued for half an hour, at which time 10 g (63 mmol) 2-chloronicotinic acid was added, and allowed to react for a further half hour. The temperature was raised to 160°C to distil off solvent and the melt was heated for two hours. At this point the melt was cooled, diluted with 50 mL water, washed with chloroform (4 by 20 mL portions), acidified with conc. HCl, and cooled. The resultant precipitate was collected and dried. The product (12.55 g, 54.3 mmol), which had a melting point of 170-171°C, was used without further purification in the next reaction. ^{1}H NMR ($CDCl_3$): 9.14δ (broad s, COOH); 8.45δ (dd, H-2, J=2 and 5 Hz); 8.32δ (dd, H-4, J=3 and 8 Hz); 7.58δ (m, H-6 and H-10); 7.44δ (m, H-7, H-8, H-9); 7.10δ (dd, H-3, J=5 and 8 Hz).

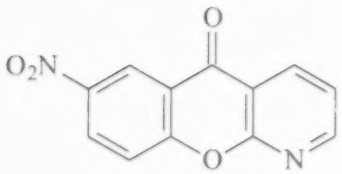


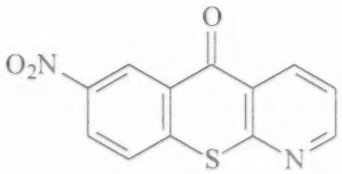
12.4 Preparation of benzothiopyrano[2,3-b]pyridine:

2,3'-Carboxypyridyl phenyl sulphide (4.0 g, 17.31 mmol) was stirred into 200 g warming PPA with a mechanical stirrer. The temperature was raised to 120°C and the reaction was stirred for 18-20 hours, at which point it was cooled and diluted with 400 mL cold water in an ice bath. The resultant thick precipitate was filtered, dried,

and used with no further purification (yield was 2.25 mg, 10.56 mmol). Melting point was 231-232°C. ^{1}H NMR ($CDCl_3$): 8.86δ (dd, H-2, J=8 and 2 Hz); 8.82δ (td, H-8, J=5, 7 and 2 Hz); 8.59δ (d, H-6, J=7.5 Hz); 7.68δ (m, H-4 and H-9, J=8, 6, 7, and 2 Hz); 7.52δ (td, H-7, J=6 and 2 Hz); 7.45δ (q, H-3, J=8 and 7 Hz).

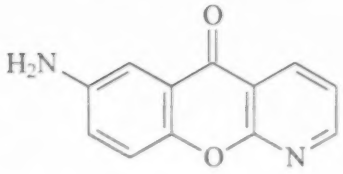
12.5 Preparation of 7-nitrobenzopyranopyridine and 7-nitrobenzothiopyranopyridine:

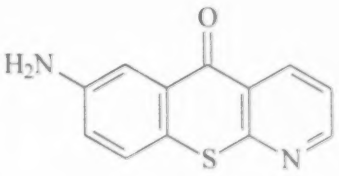




Benzopyranopyridine (or benzothiopyranopyridine) (2 g, 10.2 mmol, or 9.39 mmol respectively) was dissolved in 12 mL conc. H_2SO_4, placed in an ice bath, and cooled for 15 min. To this was added drop-wise 1.3 g (12.9 mmol) KNO_3 which had been dissolved in 2 mL of cold, conc. H_2SO_4. The solution was stirred in the ice bath for three hours at which point it was poured over crushed ice to produce a thick precipitate. The precipitate was filtered, dried, and recrystallized from large amounts of acetonitrile yielding 1.98 g (8.16 mmol) and 1.94 g (7.52 mmol) respectively. The melting point of the pyrano derivative was 237-238°C (Villani, 237-239°C), and the melting point of thiopyrano derivative was 199-201°C. ^{1}H NMR (DMSO d_6) of pyrano derivative: 9.22δ (d, H-6, J=3 Hz); 8.83δ (dd, H-2, J=4 and 2 Hz); 8.78δ (dd, H-4, J=8 and 2 Hz); 8.63δ (dd, H-8, J=9 and 3 Hz); 7.80δ (d, H-9, J=9 Hz); 7.58δ (dd, H-3, J=8, 7, 5, 4, and 3 Hz). ^{1}H NMR (DMSO d_6) of thiopyrano derivative: 9.10δ (d, H-6, J=2.8 Hz); 8.94δ (dd, H-2, J=4.9, 4.4, 2.2, 1.7 Hz); 8.78δ (dd, H-4, J=7.7, 8.3, 1.7, 2.2 Hz); 8.54δ (dd, H-8, J=8.8, 2.7 Hz); 8.19δ (d, H-9, J=8.8 Hz); 7.71δ (dd, H-3, J=8.3, 4.9 Hz).

12.6 Preparation of 7-aminobenzopyranopyridine and 7-aminobenzothiopyranopyridine:

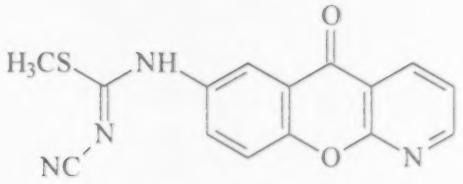




7-Nitrobenzopyranopyridine (or 7-nitrobenzothiopyranopyridine) (2 g, 8.26 mmol, or 7.75 mmol respectively) was dissolved in 30 mL methanol/HCl (1:1), to which was added 5g (26.5 mmol) $SnCl_2$. The reaction mixture was stirred at reflux for two hours, cooled, and the precipitate filtered and dried. The product was refluxed in large amounts of acetonitrile until only a grey silt was evident in the flask. The liquid was filtered hot, and the mother liquor concentrated *in vacuo* to reveal a fluffy yellow product. Yields of 1.5 g (7.02 mmol of the pyrano derivative), and 1.52 g (6.67 mmol of the thiopyrano derivative)were obtained, with melting points of 245-246°C (Villani, 246-248°C) and 235-237°C respectively. ^1H NMR ($CDCl_3$) of the pyrano derivative: 8.73δ (dd, H-2, J=2, 5, and 6 Hz); 8.68δ (d, H-4, 3 Hz); 7.51δ (dd, H-9, J=4 and 1 Hz); 7.46δ (s, H-6); 7.42δ (dd, H-3, J=8, 5 and 3 Hz); 7.16δ (dd, H-8, J=10, 9, 6, 4 and 3 Hz); 3.90δ (broad s, NH_2). ^1H NMR (DMSO d_6) of the thiopyrano derivative: 8.81δ (dd, H-2, J=2.2, 4.9, 4.4, and 1.6 Hz); 8.71δ (dd, H-4, J=2.2, 8.3, 8.3, and 2.2 Hz); 7.64δ (d, H-6, J=2.8 Hz); 7.54δ (q, H-3, J=8.3 Hz); 7.52δ (d, H-9, 8.3 Hz); 7.12δ (dd, H-8, J=2.8, 8.8, 8.7, and 2.8 Hz); 5.70δ (s, NH_2).

12.7 Modification of C-7 side chain:

12.7.1 Attempted addition of dimethyl N-cyanodithioiminocarbonate to 7-aminobenzo(thio)pyranopyridine (the following are representative of experiments performed):



i) Dimethyl N-cyanodithioiminocarbonate (700 mg, 4.8 mmol) was dissolved in 10 mL 95% ethanol to which was added 1g (4.7 mmol) of 7-aminobenzopyranopyridine. The reaction mixture was stirred at room temperature for 24 hours at which point TLC (10% methanol in chloroform) indicated no product was yet formed.

ii) Dimethyl N-cyanodithioiminocarbonate (700 mg, 4.8 mmol) was dissolved in 10 mL warming 95% ethanol to which was added 1g (4.7 mmol) 7-aminobenzopyranopyridine. The reaction was refluxed overnight but TLC (10% methanol in chloroform) indicated no product was yet formed.

iii) Dimethyl N-cyanodithioiminocarbonate (100 mg, 0.68 mmol) was dissolved in 3 mL 95% ethanol in a 5 mL screw-top glass byl to which was added 10 mg (0.05 mmol) of 7-aminobenzopyranopyridine. The lid was tightly sealed and the reaction vessel placed in a microwave on HIGH setting for five minutes. All solvent was evaporated during the heating. An unresolvable black tar resulted.

iv) Dimethyl N-cyanodithioiminocarbonate (100 mg, 0.68 mmol) was dissolved in 3 mL 95% ethanol in a 5 mL screw-top glass byl to which was added 10 mg (0.05 mmol) 7-aminobenzopyranopyridine. The lid was tightly sealed and wrapped in parafilm. The byl was microwaved on HIGH setting, but an explosion occurred after 30 seconds. Nothing was recovered.

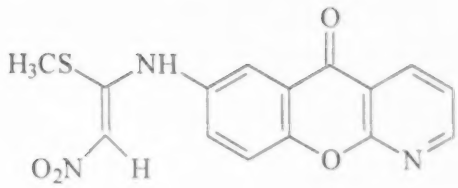
v) Dimethyl N-cyanodithioiminocarbonate (200 mg, 1.36 mmol) was dissolved in 5 mL DMF to which was added 100 mg (0.5 mmol) 7-aminobenzopyranopyridine. The reactants were stirred together at room temperature for one hour at which point TLC (acetone:hexane, 4:6) indicated that no reaction had occurredso heat (50°C) was applied for a further hour at which point TLC indicated possible product formation. The reaction mixture was diluted with 2 mL water and product extracted with chloroform (4 by 1 mL portions); however, the desired product was not recovered: product was either being formed, then destroyed by addition of water, or TLC provided erroneous information due to DMF aiding in spot migration of the reaction mixture.

vi) Dimethyl N-cyanodithioiminocarbonate (200 mg, 1.36 mmol) was dissolved in 5 mL DMF to which was added 100 mg (0.5 mmol) 7-aminobenzopyranopyridine. The reactants were stirred at 50°C for one hour at which time TLC (acetone:hexane, 4:6) indicated possible product formation. The reaction was allowed to slowly cool over one hour, then DMF was removed under high vacuum. TLC on the remaining residue indicated that the desired product was not present.

vii) Dimethyl N-cyanodithioiminocarbonate (200 mg, 1.36 mmol) was dissolved in 5 mL DMF to which was added 100 mg (0.5 mmol) 7-aminobenzopyranopyridine and the reaction mixture was heated to 90°C for 17 hours at which point TLC (acetone:hexane, 4:6) indicated possible product formation. The reaction was cooled, and the solvent removed under high vacuum; however, a repeat TLC indicated product decomposition to starting material.

viii) Dimethyl N-cyanodithioiminocarbonate (200 mg, 1.36 mmol) was dissolved in 5 mL DMF to which was added 100 mg (0.5 mmol) 7-aminobenzopyranopyridine. The reaction mixture was heated to 90°C for 5 hours at which point 20 mg (270μL, 0.27 mmol) of *n*-butylamine was added and the reaction was allowed to proceed for a further 20 hours at 80°C. TLC (20% acetone in chloroform) indicated possible product formation so the reaction was cooled, DMF removed *in vacuo* by high vacuum; however, the resultant product was unidentifiable.

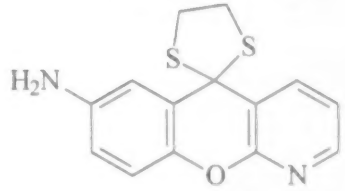
ix) Dimethyl N-cyanodithioiminocarbonate (200 mg, 1.36 mmol) was dissolved in 4 mL acetonitrile to which was added 100 mg (0.5 mmol) of 7-aminobenzopyranopyridine and the reaction mixture was stirred at room temperature for one hour. TLC (20% acetone in chloroform) indicated no reaction so the temperature was raised to 100°C for one hour. Again TLC indicated no reaction had yet occurred. The reaction was left overnight but still no product was evident so the temperature was again raised, this time to 150°C for a further 4 hours. No reaction was evident after a total reaction time of 24 hours.



x) 1,1-Bis(methylthio)-2-nitroethylene (300 mg, 1.8 mmol) was dissolved in 5 mL DMF to which was added 100 mg (0.5 mmol) of 7-aminobenzopyranopyridine and the reaction mixture was stirred at room temperature for one hour. TLC (acetone:hexane, 4:6) indicated no reaction so heat (50°C) was applied and the reaction was allowed to proceed for a further 17 hours. A weak third spot was observed on TLC so temperature was raised to 90°C for a further 3 hours at which point TLC this spot had become more intense. The reaction was cooled, and the solvent removed *in vacuo*; however, subsequent TLC indicated only starting material.

12.8 Modification of C-5 carbonyl group (the following are representative of experiments performed):

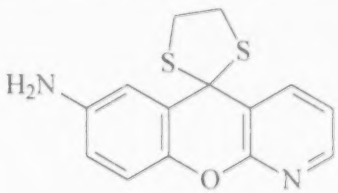
12.8.1 Attempted protection of C-5 carbonyl with ethanedithiol:



7-Aminobenzopyranopyridine (500 mg, 2.36 mmol) was dissolved in 6 mL 95% ethanol to which was added 2 mL $BF_3{\cdot}O(C_2H_5)_2$, followed by 200 µL (224.5 mg, 2.39 mmol) ethanedithiol. The reaction was stirred at room temperature for two

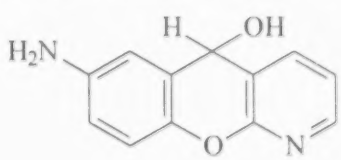
hours at which point TLC (10% methanol in chloroform) indicated possible product formation. The solvent was removed under high vacuum. The resulting product could not be identified.

12.8.2 Protection of C-5 carbonyl with ethanedithiol:



7-Aminobenzopyranopyridine (250 mg, 1.18 mmol) was stirred together with 250 mg $SOCl_2 \cdot SiO_2$ and 84 μL (94.3 mg, 1.0 mmol) ethanedithiol in 10 mL benzene at room temperature for 24 hours. TLC (10% methanol in chloroform) at this point indicated possible product formation. The silica gel was filtered and washed with 10% triethylamine in ether, the mother liquor was dried with $MgSO_4$, and solvent removed *in vacuo*. Further purification by liquid chromatography (column eluted with chloroform) revealed an amber oil. Product could not be identified but it was not the one desired.

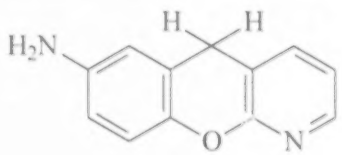
12.8.3 Reduction of C-5 carbonyl to alcohol (benzopyranopyridine):



7-Aminobenzopyranopyridine (500 mg, 2.4 mmol) was dissolved in 60 mL methanol to which was carefully added 100 mg (2.7 mmol) $NaBH_4$.The reaction was

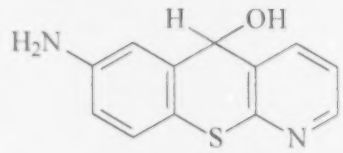
stirred at room temperature for 3 hours at which time TLC (10% methanol in chloroform) indicated no parent material remaining. The reaction was stopped by addition of acetone to precipitate a white solid ($NaBH_4$) which was filtered off. The mother liquor was concentrated *in vacuo* to reveal 400 mg (2.01 mmol) of a bright yellow solid with a melting point of 151-152°C. No further purification was performed before proceeding to the next step. ^1H NMR (DMSO d_6): 8.23δ (dd, H-2, J=1.7, 4.4, 4.9, and 2.2 Hz); 7.96δ (dd, H-4, J=2.2, 7.7, 6.6, and 1.1 Hz); 7.21δ (dd, H-3, J=4.9, 7.1, 7.1, and 4.9 Hz); 6.92δ (d, H-9, J=8.8 Hz); 6.74δ (d, H-6, J=2.8 Hz); 6.59δ (dd, H-8, J=2.2, 8.2, 8.8, and 2.8 Hz); 6.04δ (d, OH, J=5.5 Hz, exchanges with D_2O); 5.60δ (d, H-5, J=7.4 Hz, exchanges with D_2O); 4.98δ (s, NH_2, exchanges with D_2O) (Vudathala, 1990).

12.8.4 Reduction of C-5 alcohol to CH_2 (benzopyranopyridine):



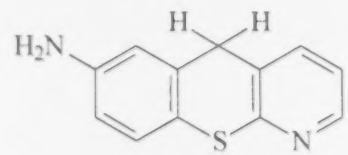
7-Aminobenzopyranopyridine-5-ol (500 mg, 2.34 mmol) was dissolved in 10 mL triflouroacetic acid and placed in an ice bath. To this was carefully added 40 mg (1.1 mmol) $NaBH_4$ and the reaction mixture was stirred for 3 hours. The solution was carefully basified, precipitate collected and recrystallized from methanol to yield 140 mg (0.702 mmol) of precipitate with a melting point of 151-152°C. ^1H NMR (DMSO d_6): 8.32δ (dd, H-2, J=1.1, 4.9, 4.9, and 1.1 Hz); 7.93δ (dt, H-4, J=1.7, 1.1, 7.7, 7.7, 1.1, and 1.7 Hz); 7.31δ (q, H-3, J=7.7 Hz); 7.11δ (d, H-9, J=8.2 Hz); 6.96δ (d, H-6, J=2.2 Hz); 6.52δ (dd, H-8, J=2.8, 8.3, 8.3, and 2.8 Hz); 6.33δ (d, H-5a, J=6.0 Hz); 5.25δ (s, NH_2); 5.18δ (d, H-5b, J=6.6 Hz).

12.8.5 Reduction of C-5 carbonyl to alcohol (benzothiopyranopyridine):



7-Aminobenzothiopyranopyridine (500 mg, 2.19 mmol) was dissolved in 20 mL THF/MeOH (1:1) to which was added 100 mg $NaBH_4$.The reaction mixture was stirred under reflux for 2 hours at which time TLC (10% methanol in chloroform) indicated all starting material was gone. The solvent was removed *in vacuo* and the residue was diluted with cold water to destroy any remaining $NaBH_4$. The beige powder was filtered, dried thoroughly, and recrystallized from 95% ethanol yielding 468 mg (2.03 mmol) of product with a melting point of 182-184°C. IR (KBr pellet): 3400-2600 cm^{-1} (O-H stretch); 1622 cm^{-1} absent (C=O no longer present).

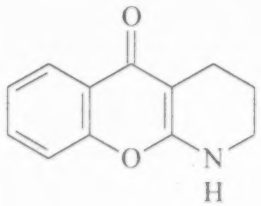
12.8.6 Reduction of C-5 alcohol to CH_2 (benzothiopyranopyridine):



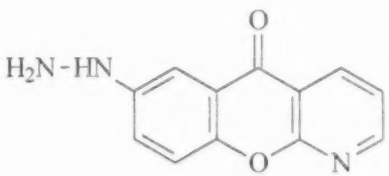
7-Aminobenzothiopyranopyridine-5-ol 250 mg (1.09 mmol) was dissolved in 5 mL triflouroacetic acid in an ice bath, to which was slowly added 100 mg $NaBH_4$. An accidental fire erupted and the reaction was stopped after five minutes by diluting with ice water and adding conc. NaOH (60%^{w}/v) until a greenish precipitate formed. The precipitate was extracted with CH_2Cl_2 and the solvent removed. The resultant 200 mg (0.935 mmol) of precipitate, melting point 108-110°C, was used with no further purification. ^{1}H NMR (DMSO d_6): 8.42δ (dd, H-2, J=1.1, 5.0, 5.0, and 1.1 Hz); 8.03δ (dt, H-4, J= 1.8, 1.1, 7.8, 7.8, 1.1, 1.8 Hz); 7.31δ (q, H-3, J=7.8 Hz); 7.09 δ

(d, H-9, J=8.2 Hz); 6.95δ (d, H-6, J=2.2 Hz); 6.51δ (dd, H-8, J=2.8, 8.2, 8.2, and 2.8 Hz); 6.32δ (d, H-5a, J=6.0 Hz); 5.25δ (s, NH_2); 5.18δ (d, H-5b, J=6.0 Hz).

12.9 Reduction of pyridine ring to 1, 2, 3, 4-tetrahydropyridine (the following is representative of experiments performed):



7-Aminobenzopyranopyridine (208 mg, 0.98 mmol) was dissolved in 150 mL 95% ethanol and placed in a pressure flask. To this was added 30 mg 10% Pd/C, sealed, and placed in the Parr Hydrogenater at 55 psi for 20 hours. The reaction was incomplete. Further attempts using different reaction times and pressures were also unsatisfactory.



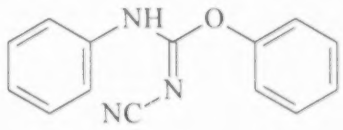
12.10 Formation of hydrazine at C-7:

7-Aminobenzopyranopyridine (300 mg, 1.4 mmol) was suspended in 8 mL conc. HCl at 0°C to which was, very slowly, added drop-wise 140 mg (2.0 mmol) $NaNO_2$ (which had been dissolved in 2 mL water). The solution turned orange and was stirred for half an hour, keeping the temperature at 0°C. The $SnCl_2$ was dissolved in 2 mL conc. HCl and was slowly added drop-wise to the solution producing a yellow precipitate. The reaction was allowed to proceed for a further 90 minutes at

which time the precipitate was filtered and washed with cold water and allowed to fully dry. A yield of 100 mg (0.44 mmol)of product with a melting point of 187-189° C resulted. ^{1}H NMR (DMSO d_6): 10.39δ (broad s, aromatic NH); 8.82δ (dd, H-2, J=2.2, 4.9, 4.4, and 1.7 Hz); 8.65δ (dd, H-4, J=7.7 and 2.2 Hz); 7.77δ (d, H-9; J=8.8 Hz); 7.69δ (d, H-6, J=2.8 Hz); 7.59δ [m, overlapping H-3, and H-8, J=7.7, 4.9, and 2.8 Hz (H-3), 4.4, 3.3, 8.8, and 2.7 Hz (H-8)]; NH_2 likely hidden under the broad DMSO peak, 3.55δ.

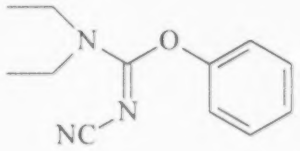
12.11 Modification of C-7 utilising trimethylaluminum as catalyst:

12.11.1 Coupling of aniline and diphenyl cyanocarbonimidate:



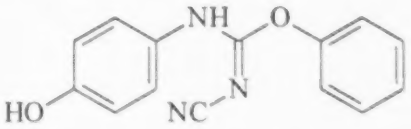
Aniline (191 µL, 2.1 mmol) was stirred together with 500 mg (2.1 mmol) diphenyl cyanocarbonimidate in 10 mL acetonitrile at reflux for 1 hour at which point TLC (10% methanol in chloroform) indicated one spot. The reaction was cooled and solvent removed *in vacuo* to reveal 213 mg (0.90 mmol) of precipitate with a melting point of 185-186°C. ^{1}H NMR (DMSO d_6): 10.57δ (broad s, NH); 7.21δ (broad m, all aromatic H's, J=7.7, 4.4, 8.3, 2.2, 7.7, 8.2, 7.1, 4.4, 7.7, 7.7 Hz).

12.11.2 Attempted coupling of diethylamine to diphenyl cyanocarbonimidate:



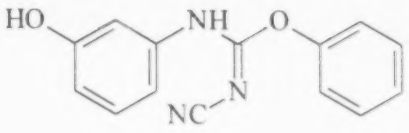
Diethylamine (219 μL, 2.1 mmol) was stirred together with 500 mg (2.1 mmol) diphenyl cyanocarbonimidate in 10 mL acetonitrile at reflux for 1 hour. The reaction was cooled and solvent removed *in vacuo*. Desired product was not recovered.

12.11.3 Coupling of *p*-hydroxyaniline to diphenyl carbonimidate:



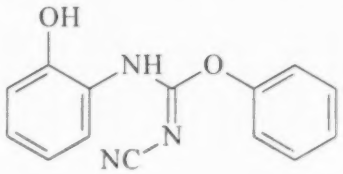
p-Hydroxyaniline (230 mg, 2.1 mmol) was stirred together with 500 mg (2.1 mmol) diphenyl cyanocarbonimidate in 10 mL acetonitrile at reflux for 1 hour at which point TLC (10% methanol in chloroform) indicated one spot in the reaction mixture different from both starting materials. The reaction was cooled and solvent removed *in vacuo* to yield 177 mg (0.70 mmol) of solid with a melting point of 155-156°C. ^{1}H NMR (DMSO d_6): 9.51δ (broad s, NH); 8.30δ (broad s, OH); 7.42δ (t, overlapping H-2 and H-4, J=7.7 and 7.7 Hz); 7.25δ (m, overlapping H-1, H-5, and H-3, J=7.7, 8.8, and 2.2 Hz); 6.76δ (dd, H-10; J=8.8, 3.3, 2.2, 2.2, and 3.3 Hz); 6.44δ (m, overlapping H-7 and H-9, J=8.8, 2.8, 2.2, 8.8, 2.7, 2.2, and 2.8 Hz).

12.11.4 Coupling of *m*-hydroxyaniline to diphenyl cyanocarbonimidate:



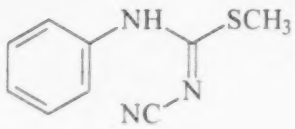
m-Hydroxyaniline (230 mg, 2.1 mmol) was stirred together with 500 mg (2.1 mmol) diphenyl cyanocarbonimidate in 10 mL acetonitrile at reflux for 1 hour at which point TLC (10% methanol in chloroform) indicated one spot in the reaction mixture different from both starting materials. The reaction was cooled and solvent removed *in vacuo*. The product was recovered as an oil. Further characterisation was not performed in light of the favourable TLC and previous ^{1}H NMR on the *p*-hydroxyaniline reaction.

12.11.5 Coupling of *o*-hydroxyaniline to diphenyl cyanocarbonimidate:



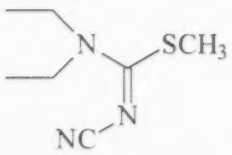
o-Hydroxyaniline (230 mg, 2.1 mmol) was stirred together with 500 mg (2.1 mmol) diphenyl cyanocarbonimidate in 10 mL acetonitrile at reflux for 1 hour at which point TLC (10% methanol in chloroform) indicated one spot in the reaction mixture different from both starting materials. The reaction was cooled and solvent removed *in vacuo*. The product recovered was an oil and was used based on TLC evidence.

12.11.6 Coupling of aniline to dimethyl N-cyanodithioiminocarbonate:



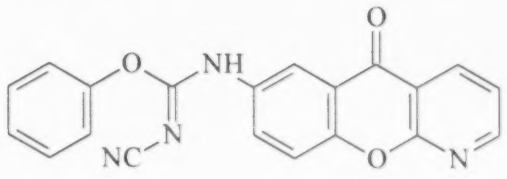
Aniline (320 mg, 3.40 mmol) was stirred together with 500 mg (3.42 mmol) dimethyl N-cyanodithioiminocarbonate in 15 mL acetonitrile at reflux for 1 hour. The reaction was cooled and solvent removed *in vacuo*. An amber oil was produced. ^1H NMR ($CDCl_3$): 7.15δ (t, H-1, H-5, J=7.7 and 8.2 Hz); 6.74δ (t, H-3, J=7.7 and 7.1 Hz); 6.67δ (d, H-2 and H-4, J=7.7 Hz); 3.68δ (broad s, NH); 2.59δ (broad s, CH_3).

12.11.7 Attempted coupling of diethylamine to dimethyl N-cyanodithioiminocarbonate:



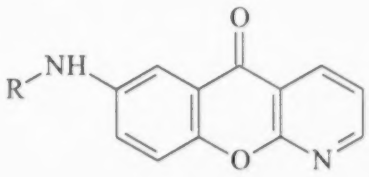
Diethylamine (153 mg, 2.1 mmol) was stirred together with 500 mg (3.42 mmol) dimethyl N-cyanodithioiminocarbonate in 15 mL acetonitrile at reflux for 1 hour. The reaction was cooled and solvent removed *in vacuo*. Purification of the resultant oil did not produce desired compound.

12.11.8 Attempted coupling of 7-aminobenzopyranopyridine to diphenyl cyanocarbonimidate



Diphenyl N-cyano carbonimide 500 mg (2.1 mmol) and 445 mg (2.1 mmol) 7-aminobenzopyranopyridine were stirred together in 10 mL acetonitrile at 100°C for 5 hours with no apparent reaction so the temperature was increased to 140°C and allowed to react overnight. The morning TLC (10% MeOH in $CHCl_3$) showed the same rf value; however, the starting material was yellow under long UV light but the reaction mixture spot was fluorescent. Several other fluorescent spots were also present but attempted purification of the reaction from MeOH did not yield the expected compound.

12.12 Attempted coupling of above amines (R) with 7-aminobenzopyranopyridine (the following is representative of experiments performed):

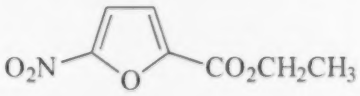


7-aminobenzopyranopyridine (250 mg, 1.18 mmol) was stirred together in a slurry with 5 mL dichloroethane under nitrogen for ten minutes. To this was carefully added 1.24 mL trimethylaluminum at 0°C. The reaction was allowed to warm to room

temperature and was stirred for half an hour. At this point the reaction product was dissolved in 1 mL dichloroethane and the reaction was warmed to 60°C for two hours. The reaction mixture was then cooled to room temperature, diluted with CH_2Cl_2, and neutralised with 10% $NaHCO_3$. The non-aqueous layer was evaporated leaving a dark yellow oil which was purified on a column eluted with 10% MeOH in $CHCl_3$. Work-up gave several fractions and the last two fractions were combined and solvent removed *in vacuo*. A yield of 40 mg bright red crystalline product (10%, 0.11 mmol) with a melting point of 65°C was recovered. ^{1}H NMR (DMSO d_6): 6.85δ (d, J=7.7 Hz); 6.77δ (t, J=7.1 and 8.3 Hz); 6.64δ (m, J=9.9, 7.1, 7.1, and 7.2 Hz). NMR pattern did not fit that expected.

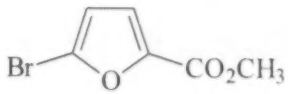
13.0 MODIFICATIONS TO FURAN STARTING MATERIAL:

13.1 Preparation of ethyl 5-nitrofuran-2-carboxylate:



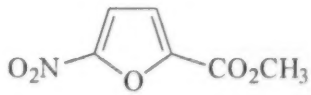
5-Nitro-2-furoic acid (500 mg, 3.18 mmol) was dissolved in 50 mL 95% ethanol, to which was added 5 mL conc. H_2SO_4. The reaction was stirred at 80°C for 18-20 hours at which point the reaction was cooled, and the ethanol was removed *in vacuo* leaving some H_2SO_4. This was then diluted with water and the precipitate was filtered and dried. No further purification was necessary. Yield was 460 mg (2.5 mmol), and the melting point was 95°C. ^{1}H NMR ($CDCl_3$): 7.35δ (d, H-3, J=3.8); 7.30δ (d, H-4, J=3.8 Hz); 4.50δ (q, CH_2, J=7.2 Hz); 1.36δ (t, CH_3, J=7.2 Hz). IR (KBr pellet): 1726 cm^{-1} (C=O); 1532 cm^{-1} (NO_2); 1351 cm^{-1} (NO_2).

13.2 Preparation of methyl 5-bromofuran-2-carboxylate:



5-Bromo-2-furoic acid (1.0 g, 5.23 mmol) was dissolved in 150 mL methanol and warmed to 50°C at which time 2 mL of conc. H_2S0_4 was added. The temperature was increased to 100°C and the reaction was allowed to proceed for 3.5 hours. Progress of the reaction was monitored by TLC (4:6 acetone:hexane). The reaction was allowed to cool and the solvent was removed *in vacuo*. The final product was washed with cold methanol and dried thoroughly. The yield was 325 mg (1.58 mmol) of crystals with a melting point of 58°C. IR (KBr pellet): 1710 cm^{-1} (C=O); 1580 cm^{-1} (O-CH_3).

13.3 Preparation of methyl 5-nitrofuran-2-carboxylate:

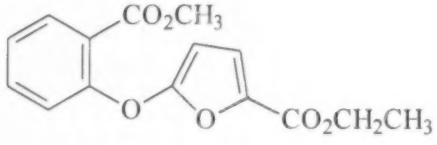


5-Nitro-2-furoic acid (2.0 g, 12.7 mmol) was dissolved in 200 mL methanol and acidified to pH 0.5 with 5 mL conc. H_2S0_4. The reaction was heated to reflux on a water bath and allowed to react for 6 hours at which point TLC (chloroform) indicated no remaining starting material. The reaction was cooled, solvent removed *in vacuo*, and the H_2S0_4 residue was diluted with cold water which yielded the precipitate which was used with no further purification necessary. The yield was 1.81 g (10.6 mmol) of pale yellow crystals with a melting point of 68-70°C. ^1H NMR

($CDCl_3$): 7.35δ (d, H-3, J=3.8 Hz); 7.31δ (d, H-4, J=3.8 Hz); 3.99δ (s, CH_3). IR (KBr pellet): 1728 cm^{-1} (C=O); 1532 cm^{-1} (NO_2); 1359 cm^{-1} (NO_2).

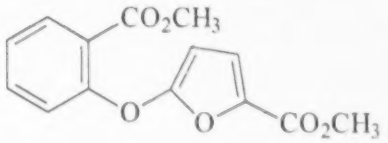
13.4 Addition of methyl salicylate to furan esters:

13.4.1 Addition of methyl salicylate to ethyl 5-nitrofuran-2-carboxylate:



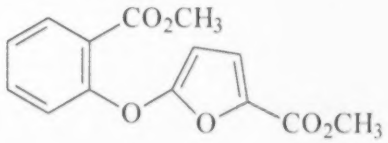
Methyl salicylate (175 μL, 1.36 mmol) was dissolved in 5 mL DMSO to which was carefully added 210 mg (168 mg pure NaH, 7.0 mmol) NaH (80% dispersed in oil), warmed to 100°C and allowed to react for half an hour. To this was added drop-wise over half an hour 261 mg (1.41 mmol) ethyl 5-nitrofuran-2-carboxylate which had been dissolved in 2 mL DMSO. The reaction was allowed to proceed for a further 2 hours then cooled. The reaction mixture was poured over ice water and extracted with benzene (4 by 10 mL portions). The benzene layer was then washed with 10%^{w}/v KOH, dried with $MgSO_4$, and then the solvent removed *in vacuo* to reveal an amber oil. The product was further purified by liquid chromatography column eluted with chloroform. The yield was 890 mg (3.22 mmol; 60%) of an amber oil. ^1H NMR ($CDCl_3$): 7.96δ (dd, H-8, J=7.7 and 1.7 Hz); 7.55δ (td, H-6, J=13.8, 1.9, 5.8, 13.7, 8.0, and 1.7 Hz); 7.30δ (t, H-7, J=7.7 Hz); 7.15δ [m, overlapping H-5 and H-1, J=6.6 Hz (H-5) and 3.6 Hz (H-1)]; 5.42δ (d, H-10, J=3.6 Hz); 4.34δ (q, CH_2, J=7.1, 7.2, and 7.1 Hz); 3.86δ (s, CH_3 of ester); 1.36δ (t, CH_3, J=7.1 and 6.9 Hz). IR (neat): doublet centred at 1720 cm^{-1} (C=O of methyl and ethyl esters): methyl salicylate shows 1679 cm^{-1} (C=O, ester).

13.4.2 Attempted addition of methyl salicylate to methyl 5-bromofuran-2-carboxylate (the following is representative of experiments performed):



Sodium metal (76 mg, 3.3 mmol) was dissolved in 800μL (6.2 mmol) methyl salicylate while being warmed to 100°C. To this solution was added 300 mg (1.5 mmol) 5-bromo-2-furoic acid and the reaction was allowed to proceed. Reaction progress was monitored by TLC (1:9 acetone:hexane). The reaction was left to proceed over the weekend but the desired product could not be isolated.

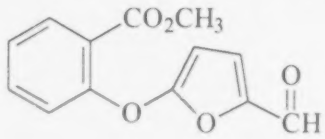
13.4.3 Addition of methyl salicylate to methyl 5-nitrofuran-2-carboxylate:



Methyl salicylate (1033 μL, 8.0 mmol) was dissolved in 5 mL DMSO and gently warmed to 100°C as 480 mg (384 mg pure NaH, 16 mmol) NaH (80% dispersed in oil) was slowly added. To this 1.0 g (5.35 mmol) methyl 5-nitrofuran-2-carboxylate dissolved in 2 mL of DMSO was added over an hour. The reaction was monitored by TLC (chloroform) and after one hour the reaction was diluted with ice water, extracted with chloroform (4 by 10 mL portions), and rinsed with 10%^{W}/v KOH. The solvent was removed *in vacuo* and the product was purified on a silica column with chloroform elution. A pale amber oil weighing 890 mg (3.22 mmol) resulted. ^{1}H NMR ($CDCl_3$): 7.96δ (dd, H-8, J=7.7 and 1.7 Hz); 7.54δ (td, H-6, J=7.7, 1.7, 7.1 and 8.3 Hz); 7.30δ (td, H-7, J=7.7, 8.3, and 1.1 Hz); 7.15δ [m, overlapping H-5 and H-1, J=1.1, 7.2 Hz (H-5), and 3.8 Hz (H-1)]; 5.41δ (d, H-10, J=3.3 Hz); 4.34δ

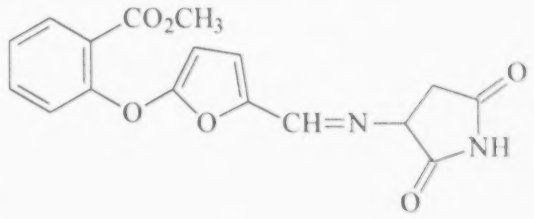
(q, CH_2, J=7.1 Hz); 3.86δ [d, CH_3 of methyl ester, J=1.7 Hz (long range coupling to H-8)]; 1.36δ (t, CH_3 of ethyl ester, J=7.1 Hz). IR (neat): broad peak centred on 1720 cm^{-1} (C=O of methyl and ethyl esters). CI-MS (NH_3): M+18=294 amu; M+H=277 amu; M^+=276 amu.

13.4.4 Addition of methyl salicylate to 5-nitro-2-furaldehyde:



NaH [(80% dispersed in oil) 800 mg, 640 mg pure NaH, 26.7 mmol] was stirred in 5 mL warming DMSO to which was carefully added 1695 μL (13.2 mmol) methyl salicylate and stirred for half an hour. 5-Nitro-2-furaldehyde (741 μL, 100 mg, 7.1 mmol) was dissolved in 2 mL DMSO and carefully added drop-wise over a period of about one hour. The reaction was stirred at 100°C for three hours at which point it was cooled, diluted with ice water, extracted with chloroform, and the non-aqueous layer washed with 10%^{w}/v KOH. The chloroform layer was dried with $MgSO_4$ and the solvent was removed *in vacuo* to reveal an amber oil which was then further purified on silica column, eluted with chloroform. A yield of 750 mg (3.1 mmol) resulted. ^{1}H NMR ($CDCl_3$): 9.39δ (s, aldehyde proton); 8.01δ (dd, H-8, J=1.7, and 7.7 Hz); 7.60δ (complex td, H-6, J=1.9, and 8.2 Hz); 7.37δ (td, H-7, J=1.1, 7.7, 7.4, 0.8, 7.4, 7.7, and 1.1 Hz); 7.25δ (dd, H-5, J=0.8, and 8.2 Hz); 7.22δ (d, H-3, J=3.8 Hz); 5.45δ (d, H-4, J=3.8 Hz), 3.84δ (s, CH_3 of methyl ester). IR (neat): 1720 cm^{-1} (aldehyde C=O); 1671 cm^{-1} (methyl ester C=O); NO_2 peaks at 1532 and 1351 cm^{-1} are absent. EI-MS (low resolution): $M^{+\cdot}$=246 amu ($C_{13}H_{10}O_5$); base peak 126 amu ($C_6H_6O_3$).

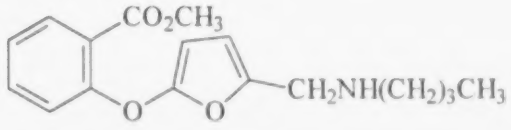
13.4.5 Attempted addition of methyl salicylate to nitrofurantoin (the following is representative of experiments performed):



NaH [(80% dispersed in oil), 400 mg, 320 mg, 13.3 mmol] was stirred in 2.5 mL warming DMSO to which was carefully added 847 µL (6.6 mmol) of methyl salicylate. The mixture was allowed to react for half an hour. Nitrofurantoin (950 mg, 4.4 mmol) was dissolved in 2.5 mL and carefully added drop-wise to the reaction over about one hour. The reaction was stirred at 80°C for 24 hours at which point the reaction was cooled, diluted with ice water, extracted with chloroform, and the non-aqueous layer washed with 10%^{W}/v KOH. The chloroform layer was dried with $MgSO_4$, and solvent removed *in vacuo* by roto-vap to reveal an amber oil which was further purified on a silica column eluted with chloroform. The resultant product was not the desired one and could not be identified.

13.5 Attempted modification of 5-(2'-methoxycarbonylphenoxy)furan-2-aldehyde (the following are representative of experiments performed):

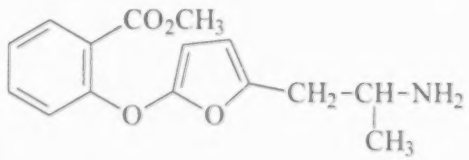
13.5.1 Attempted addition of *n*-butylamine:



5-(2'-Methoxycarbonylphenoxy)furan-2-aldehyde (140 mg, 0.57 mmol) was stirred with 100 µL (1.0 mmol) *n*-butylamine in 1 mL methanol at reflux for one hour

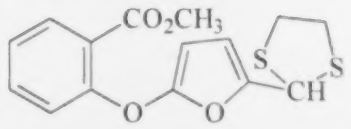
at which point the reaction was cooled slightly, and diluted with 1 mL water. To this, 40 mg (0.65 mmol) $NaCNBH_3$ was added. The reaction was allowed to proceed for a further hour at reflux at which point it was cooled, washed with chloroform, and aqueous layer removed *in vacuo*. TLC (10% chloroform in methanol) of the chloroform layer revealed a multitude of spots: TLC of the residue also indicated a multitude of products produced, likely indicating decomposition of the furan ring.

13.5.2 Attempted addition of nitroethane:



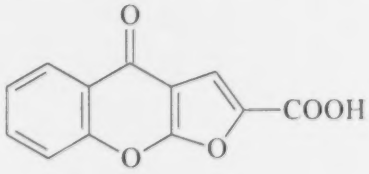
5-(2'-Methoxycarbonylphenoxy)furan-2-aldehyde (200 mg, 0.81 mmol) was stirred together with 43.7 µL (0.81 mmol) nitroethane in 2 mL methanol in an ice bath at ~10°C for five minutes. To this was slowly added 2 mL cold 40%^{w}/v NaOH, the reaction was slowly warmed to 15-20°C, and stirred for 15 minutes. To this was added 10 mL ice water and then 5 mL 6M HCl. This was washed with ether, but no significant transfer to the non-aqueous layer was noted. The aqueous layer was then removed *in vacuo* by roto-vap to reveal an off-white precipitate. The precipitate was dissolved in 2 mL 6M HCl and 2 mL 95% ethanol at room temperature to which was added 500 mg tin. The reaction was stirred for half an hour at which point cold 40%^{w}/v NaOH was added, the tin filtered, and the water removed *in vacuo*. No desired product could be isolated. A wash of the precipitate with chloroform was performed; however, again no desired product could be isolated.

13.5.3 Attempted protection of C-2 aldehyde:



5-(2'-Methoxycarbonylphenoxy)furan-2-aldehyde (250 mg, 1.0 mmol) was dissolved in 10 mL benzene at room temperature to which was added 250 mg $SOCl_2 \cdot SiO_2$ followed by 84 μL (103 mg, 1.1 mmol) dithioethane. The reaction was stirred for 24 hours at which point the silica was filtered and washed with ether-10% triethylamine and dried with $MgSO_4$. The solvent was removed *in vacuo*, and further purified on a silica column eluted with chloroform. An amber oil resulted but NMR revealed that the desired reaction had not occurred.

13.6 Attempted cyclization to furo[2,3-b]chromone ring system (the following are representative of experiments performed):



i) Ethyl 5-(2'-methoxycarbonylphenoxy)furan-2-carboxylate (100 mg, 0.344 mmol) was dissolved in 16 mL benzene, added to 16 g of PPA, and stirred by mechanical stirrer at 180°C. The reaction time was about 24 hours. The temperature was probably too high and multiple spots were produced on TLC (chloroform).

ii) Ethyl 5-(2'-methoxycarbonylphenoxy)furan-2-carboxylate (150 mg, 0.517 mmol) was dissolved in 16 mL benzene and added to 16 g PPA. The reaction was

stirred by mechanical stirrer at 120°C for 24 hours at which time TLC (chloroform) indicated possible product formation. The reaction was stopped with the addition of water and allowed to cool to room temperature. Potential product was extracted with chloroform (4 by 10 mL portions), solvent evaporated *in vacuo*, and the product was purified by preparative TLC. The reaction yielded 30 mg precipitate on which ^{1}H NMR was inconclusive: despite purification by preparative TLC, the spectrum was noisy and gave no clear indication of the product formed.

iii) Methyl 5-(2'-methoxycarbonylphenoxy)furan-2-carboxylate (180 mg, 0.65 mmol) was dissolved in 17 mL benzene and heated to 120°C in 15.75 g PPA for 24 hours. The reaction was cooled with the addition of ice water, washed with chloroform (4 by 10 mL portions), and the solvent was removed *in vacuo*. The resulting oil was purified on a silica column with chloroform elution. The components could not be identified, but did not appear to be the desired product.

iv-a) Methyl 5-(2'-methoxycarbonylphenoxy)furan-2-carboxylate (500 mg, 1.81 mmol) was stirred at reflux in 10 mL 25%^{w}/v NaOH overnight. The solution was one phase at this point and was acidified to pH 0.5 with conc. HCl. A cream coloured precipitate was filtered and allowed to fully dry. A yield of 353 mg (1.4 mmol) cream coloured crystals with a melting point of 167°C (sharp) resulted. ^{1}H NMR (DMSO d_6): 10.53δ (broad s, COOH); 7.91δ (dd, H-8, J=1.7, and 1.7 Hz); 7.67δ (td, H-6, J=1.7, 1.1, and 1.7 Hz); 7.41δ (td, H-7, J=1.1, 1.1 Hz); 7.30δ (d, H-5, J=7.7 Hz); 7.20δ (d, H-1, J=3.9Hz); 5.55δ (d, H-9, J=3.3 Hz). IR (KBr

pellet): broad 2483-3246 cm^{-1} (carboxylic acid O-H); doublet centred on 1687 cm^{-1} (C=O stretch of two carbonyls).

iv-b) The 5-(2'-carboxyphenoxy)furan-2-carboxylic acid (350 mg, 1.4 mmol) was then stirred together with 5 mL benzene and 5 g PPA and warmed to 100°C for 6 hours. The reaction was cooled, diluted with 100 mL ice water and stirred further overnight. The benzene layer was washed with water, dried with $MgSO_4$, and solvent evaporated *in vacuo*. The resulting oil was purified on a silica column with chloroform elution. The purified oil produced was not the desired product, but rather a mixture whose main component was free salicylic acid. ^1H NMR ($CDCl_3$); 10.38δ (broad s, COOH); 7.94δ (dd, H-6, J=1.7, and 7.7 Hz); 7.54δ (td, H-4, J=2.2, 8.3, 1.7, 7.1, 1.6, and 7.1 Hz); 6.98δ (complex m, overlapping H-3 and H-5).

v) 5-(2'-carboxyphenoxy)furan-2-carboxylic acid (600 mg, 2.4 mmol) and 8 mL toluene were stirred together with 8.7 g PPA at 120°C for one hour at which time TLC (chloroform) indicated a reaction was occurring and possible cleavage was taking place. The reaction was then cooled, diluted with water, and stirred for 18 hours as was outlined in Kuo *et al* (1989). TLC then indicated a number of spots (at least 6) and separation was minimal. Crystallisation from methanol was attempted to separate the lower spots which were suspected to contain the desired product. Crystallisation occurred leaving a solid with the more polar substances dissolved. The methanol was decanted and solvent removed *in vacuo* leaving an unidentifiable mixture.

vi) The 5-(2'-carboxyphenoxy)furan-2-carboxylic acid (100 mg, 0.4 mmol) was suspended in 5 mL chloroform and warmed to 80°C. To this was slowly added 58.4 μL (0.8 mmol) thionyl chloride. The mixture was allowed to react for one and a half hours. At this point 117 mg (0.88 mmol) $AlCl_3$ was added and the heat was turned off. TLC (chloroform) over the next half hour revealed spot migration similar to that seen in the reaction with PPA so the reaction was stopped. Cold water was added to the mixture which was then allowed to cool. An off white precipitate was filtered and thoroughly dried. NMR showed no significant change (i.e. the proton at 5.5δ was still present indicating cyclization did not occur). Filtered precipitate yielded 75 mg with a melting point of 161°C. ^1H NMR indicated the product was essentially identical to furanoic acid.

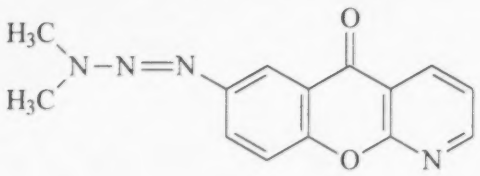
vii) 5-(2'-Carboxyphenoxy)furan-2-carboxylic acid chloride (75 mg, 0.26 mmol) and 140 mg (1.0 mmol) $AlCl_3$ were stirred together and then 4 mL CS_2 was added. No evolution of gas noted upon addition of the solvent so heat was applied. The reaction was stirred overnight but the solvent evaporated. The white solid produced was not identifiable (melting point >185°C).

viii) 5-(2'-Carboxyphenoxy)furan-2-carboxylic acid chloride (125 mg, 0.44 mmol) and 138 mg (1.0 mmol) $AlCl_3$ were stirred together in 100 mL nitrobenzene (which had been purified and dried) at 100°C. TLC (10% methanol in chloroform) was difficult to perform. Small portions of the reaction mixture were removed, hydrolysed with 40%^{W}/v NaOH and acidified with conc. HCl in order to see the compound without the nitrobenzene interfering. The reaction was allowed to proceed overnight at which time TLC indicated a reaction had occurred. At this

point the reaction was cooled and worked up as for the TLC preparation. The water was removed *in vacuo* leaving a large quantity of NaCl and potentially the desired product. The dry crystals were washed well with chloroform, solvent collected and then removed *in vacuo* by roto-vap to leave a small amount of beige precipitate which was recrystallized from ethanol-water. The remaining soft fluffy crystals were collected and thoroughly dried. A 100 mg quantity resulted with a melting point of 151-153°C. ^{1}H, decoupling, and ^{13}C NMR as well as MS (low and high resolution EI, and CI) were indicative of free salicylic acid (reported mp of salicylic acid 157-159°C, Merck). ^{1}H NMR ($CDCl_3$): 11.39δ (broad s, COOH?); 10.37δ (s, H-3?); 7.95δ (dd, H-5, J=8.3, 6.6, 1.7, and 1.7 Hz); 7.55δ (td, H-7, J=8.8, 7.1, 6.6, 2.2, and 1.7 Hz); 6.99δ (m, overlapping H-6 and H-8, J=8.8, 8.8, 1.1, 7.1, and 7.1 Hz). ^{13}C NMR ($CDCl_3$): 6 carbons present: 174.97δ (C=O); 162.24δ (CH-2, likely attached to OH); 137.03δ (CH-6); 130.98δ (CH-3); 119.60 δ (CH-4); 117.87δ (CH-5); 111.28δ (CH-1). EI MS (high resolution): M^{+}=138.032 amu ($C_7H_6O_3$) whereas a molecular ion of 202 amu was predicted; base peak m/z=120.021 amu ($C_7H_4O_2$). Low resolution CI-MS (NH_3): M^{+} m/z=273 amu. IR (KBr pellet): broad peak, 3200-2400 cm^{-1} (OH stretch of COOH), 1663 and 1622 cm^{-1} (C=O stretch of C-4 carbonyl, and C=O of COOH). Reported values for C=O groups were 1710 and 1670 cm^{-1} respectively.

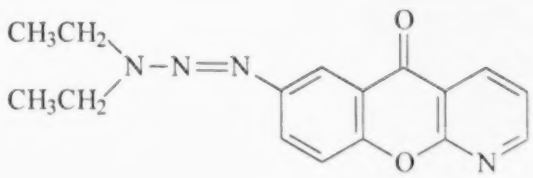
14.0 PREPARATION OF 7-DIAZAMINO COMPOUNDS:

14.1 Preparation of 7-(diazadimethylamino)-benzopyranopyridine:



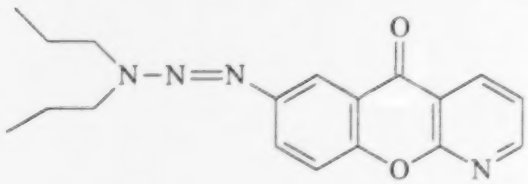
7-Aminobenzopyranopyridine (500 mg, 2.4 mmol) was dissolved in 10 mL 50%^{V}/v H_2SO_4 and cooled in an ice bath to 5-0°C. To this was slowly added 620 mg (9.0 mmol) $NaNO_2$ which had been dissolved in 1.25 mL H_2O. The reaction was stirred in the ice bath for two hours at which time 652 mg (8 mmol) dimethylamine was added slowly. The reaction was allowed to proceed for another 1/2 hour, still in the ice bath, at which time the reaction was basified with 40%^{W}/v NaOH. The resultant precipitate was filtered and the filtrate washed with ethyl acetate. The precipitate was also washed thoroughly with ethyl acetate and filtrate collected. The filtrates and ethyl acetate washes were combined and solvent removed *in vacuo* to leave 485 mg (1.81 mmol) of precipitate with a melting point of 199-201°C (acetonitrile). ^{1}H NMR ($CDCl_3$): 8.72δ (m, overlapping H-2 and H-4, J=2.2, 7.7, 2.8, and 1.7 Hz); 8.26δ (d, H-6, J=2.7 Hz); 7.88δ (dd, H-8, J=2.2, 8.8, 9.3, and 2.8 Hz); 7.56δ (d, H-9, J=8.8 Hz); 7.41δ (dd, H-3, J=4.9, 7.7, 7.7 and 4.9 Hz); 3.44δ (broad s, gem N-dimethyl). IR (KBr pellet): 3425 cm^{-1}(aromatic amine stretch); 2985 cm^{-1}(aliphatic C-H stretch); 2200-2400 cm^{-1} N-N=N stretch missing; 1669 cm^{-1}(aromatic ketone stretch). EI-MS: M^{+}=268.12 amu ($C_{14}H_{12}N_4O_2$); base peak m/z=196 amu ($C_{12}H_6NO_2$).

14.2 Preparation of 7-(diazadiethylamino)-benzopyranopyridine:



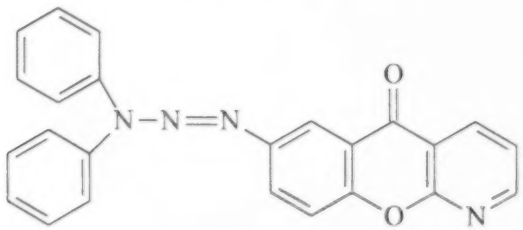
7-Aminobenzopyranopyridine (500 mg, 2.4 mmol) was dissolved in 10 mL 50%^{v}/v H_2SO_4 and cooled in an ice bath to 5-0°C. To this was slowly added 620 mg (9.0 mmol) $NaNO_2$ which had been dissolved in 1.25 mL H_2O. The reaction was stirred in the ice bath for two hours at which time 837 µL (584 mg, 8.0 mmol) diethylamine was added slowly. The reaction was allowed to proceed for another 1/2 hour, still in the ice bath, at which time the reaction was basified with 40%^{w}/v NaOH. The resultant precipitate was filtered and the filtrate washed with ethyl acetate. The precipitate was also washed thoroughly with ethyl acetate and filtrate collected. The filtrates and ethyl acetate washes were combined and solvent removed *in vacuo* to leave 573 mg (1.93 mmol) of the final product with a melting point of 131-132°C (acetoniltrile). ^{1}H NMR ($CDCl_3$): 8.72δ (td, overlapping H-2 and H-4, J=2.2, 4.9, 2.2, 5.5, and 2.2 Hz); 8.24δ (d, H-6, J=2.8 Hz); 7.88δ (dd, H-8, J=2.8, 8.8, 8.8, and 2.8 Hz); 7.56δ (d, H-9, J=8.8 Hz); 7.41δ (dd, H-3, J=4.9, 7.7, 7.7, and 4.9 Hz); 3.79δ (q, two CH_2, J=7.2, 14.3, 14.3 and 7.2 Hz); 1.26δ (broad s, two CH_3). IR (KBr pellet): 3429 cm^{-1}(aromatic amine stretch); 2918 cm^{-1}(aliphatic C-H stretch); 2358 cm^{-1} (N-N=N stretch); 1660 cm^{-1} (aromatic ketone stretch). EI-MS: M^+=296.15 amu ($C_{16}H_{16}N_4O_2$); base peak m/z=196 amu ($C_{12}H_6NO_2$).

14.3 Preparation of 7-(diazadipropylamino)-benzopyranopyridine:



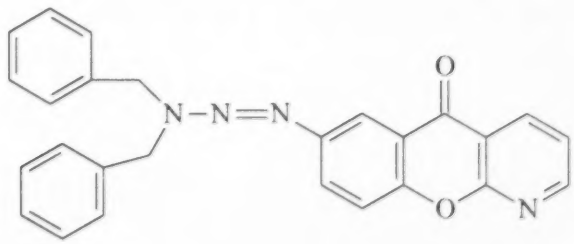
7-Aminobenzopyranopyridine (500 mg, 2.4 mmol) was dissolved in 10 mL 50%^{v}/v H_2SO_4 and cooled in an ice bath to 5-0°C. To this was slowly added 620 mg (9.0 mmol) $NaNO_2$ which had been dissolved in 1.25 mL H_2O. The reaction was stirred in the ice bath for two hours at which time 808 mg (1.1 mL, 8.0 mmol) dipropylamine was added slowly. The reaction was allowed to proceed for another 1/2 hour, still in the ice bath, at which time the reaction was basified with 40%^{w}/v NaOH. The resultant precipitate was filtered and the filtrate washed with ethyl acetate. The precipitate was also washed thoroughly with ethyl acetate and filtrate collected. The filtrates and ethyl acetate washes were combined and solvent removed *in vacuo* to leave 390 mg (1.2 mmol) of precipitate with a melting point of 92-94°C (acetoniltrile). ^{1}H NMR ($CDCl_3$): 8.74δ (m, overlapping H-2 and H-4, J=2.2, 7.1, 4.9, and 1.7 Hz); 8.23δ (d, H-6, J=2.2 Hz); 7.88δ (dd, H-8, J=2.2, 8.8, 9.3, and 2.8 Hz); 7.56δ (d, H-9, J=6.6 Hz); 7.41δ (dd, H-3, J=4.9, 7.7, 7.1, and 4.4 Hz); 3.69δ (t, CH_2-N, J=7.1, and 7.7 Hz); 1.73δ (q, CH_2, J=8.0, 13.7, and 7.1 Hz); 0.96δ (t, CH_3, J=7.2, and 7.7 Hz). IR (KBr pellet): 3429 cm^{-1} (aromatic amine stretch); 2958 cm^{-1} (aliphatic C-H stretch); 2361 cm^{-1} (N-N=N stretch); 1662 cm^{-1} (aromatic ketone stretch). EI-MS: M^+=324.19 amu ($C_{18}H_{20}N_4O_2$); base peak m/z=196 amu ($C_{12}H_6NO_2$).

14.4 Attempted preparation of 7-(diazadiphenylamino)-benzopyranopyridine:



7-Aminobenzopyranopyridine (500 mg, 2.4 mmol) was dissolved in 10 mL 50%^{V}/v H_2SO_4 and cooled in an ice bath to 5-0°C. To this was slowly added 620 mg (9.0 mmol) $NaNO_2$ which had been dissolved in 1.25 mL H_2O. The reaction was stirred in the ice bath for two hours at which time 1352 mg (8.0 mmol) diphenylamine was added slowly. The reaction was allowed to proceed for another 1/2 hour, still in the ice bath, at which time the reaction was basified with 40%^{W}/v NaOH. The resultant precipitate was filtered and the filtrate washed with ethyl acetate. The precipitate was also washed thoroughly with ethyl acetate and filtrate collected. The filtrates and ethyl acetate washes were combined and solvent removed *in vacuo* to leave a thick black oil which could not be identified despite numerous attempts to purify.

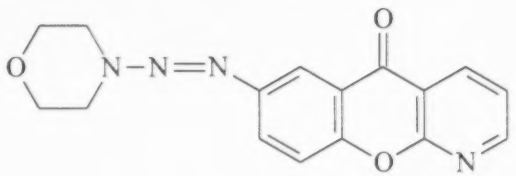
14.5 Attempted preparation of 7-(diazadibenzylamino)-benzopyranopyridine:



7-Aminobenzopyranopyridine (500 mg, 2.4 mmol) was dissolved in 10 mL 50%^{V}/v H_2SO_4 and cooled in an ice bath to 5-0°C. To this was slowly added 620 mg

(9.0 mmol) $NaNO_2$ which had been dissolved in 1.25 mL H_2O. The reaction was stirred in the ice bath for two hours at which time 1.5 mL (1.58 mg, 8.0 mmol) dibenzylamine was added slowly. The reaction was allowed to proceed for another 1/2 hour, still in the ice bath, at which time the reaction was basified with 40%^{W}/v NaOH. The resultant precipitate was filtered and the filtrate washed with ethyl acetate. The precipitate was also washed thoroughly with ethyl acetate and filtrate collected. The filtrates and ethyl acetate washes were combined and solvent removed *in vacuo* to leave a black oil which after repeated attempts could not be purified.

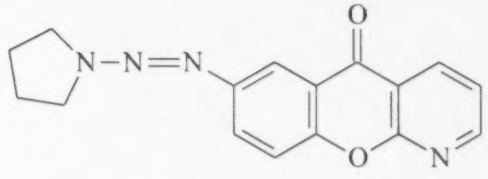
14.6 Preparation of 7-(diazamorpholino)-benzopyranopyridine:



7-Aminobenzopyranopyridine (500 mg, 2.4 mmol) was dissolved in 10 mL 50%^{V}/v H_2SO_4 and cooled in an ice bath to 5-0°C. To this was slowly added 620 mg (9.0 mmol) $NaNO_2$ which had been dissolved in 1.25 mL H_2O. The reaction was stirred in the ice bath for two hours at which time 700 µL (696 mg, 8.0 mmol) morpholine was added slowly. The reaction was allowed to proceed for another 1/2 hour, still in the ice bath, at which time the reaction was basified with 40%^{W}/v NaOH. The resultant precipitate was filtered and the filtrate washed with ethyl acetate. The precipitate was also washed thoroughly with ethyl acetate and filtrate collected. The filtrates and ethyl acetate washes were combined and solvent removed *in vacuo* to leave 100 mg (0.32 mmol) of precipitate with a melting point of 170°C (acetonitrile). ^{1}H NMR ($CDCl_3$): 8.75δ [m, H-2 and H-4, overlapped, J=2.2, 4.9, 4.9,

and 2.2 Hz (H-2); J=3.9, 7.8, 6.0, and 2.2 Hz (H-4)]; 8.33δ (d. H-6, J=2.7 Hz); 7.93δ (dd, H-8, J=2.8, 8.8, 8.8, and 2.8 Hz); 7.63δ (d, H-9, J=8.8 Hz); 7.46δ (dd, H-3, J=4.9, 7.7, 7.7, and 4.9 Hz); 3.88δ (s, morpholino H's). IR (KBr pellet): 3422 cm^{-1} (aromatic amine stretch); 2972 cm^{-1} (aliphatic C-H stretch); 2347 cm^{-1} (weak N-N=N stretch); 1662 cm^{-1} (aromatic ketone stretch). EI-MS: M^{+}=310.12 amu ($C_{16}H_{14}N_4O_2$); base peak m/z=196 amu ($C_{12}H_6NO_2$).

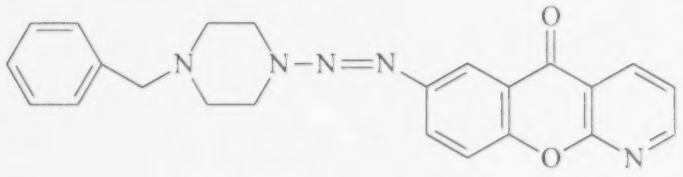
14.7 Preparation of 7-(diazapyrrolidino)-benzopyranopyridine:



7-Aminobenzopyranopyridine (500 mg, 2.4 mmol) was dissolved in 10 mL 50%^{V}/v H_2SO_4 and cooled in an ice bath to 5-0°C. To this was slowly added 620 mg (9.0 mmol) $NaNO_2$ which had been dissolved in 1.25 mL H_2O. The reaction was stirred in the ice bath for two hours at which time 667 (568 mg, 8.0 mmol) pyrrolidine was added slowly. The reaction was allowed to proceed for another 1/2 hour, still in the ice bath, at which time the reaction was basified with 40%^{W}/v NaOH. The resultant precipitate was filtered and the filtrate washed with ethyl acetate. The precipitate was also washed thoroughly with ethyl acetate and filtrate collected. The filtrates and ethyl acetate washes were combined and solvent removed *in vacuo* to leave 480 mg (1.63 mmol) of precipitate with a melting point of 165°C (acetonitrile). ^1H NMR ($CDCl_3$): 8.71δ (m, overlapping H-2 and H-4, J=2.2, 2.2, 4.9, and 7.1 Hz); 8.23δ (d, H-6, J=2.2 Hz); 7.86δ (dd, H-8, J=2.7, 9.3, 8.8, and 2.2 Hz); 7.55δ (d, H-9, J=7.1 Hz); 7.41δ (dd, H-3, J=4.4, 7.7, 7.7, and 4.4 Hz); 3.91δ (broad s, two H-1');

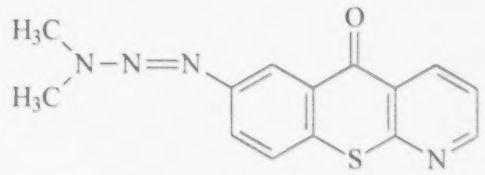
3.71δ (broad s, two H-4'); 2.03δ (t, four protons: two H-2' and two H-3', J=2.2, and 1.7 Hz). IR (KBr pellet): 3429 cm^{-1} (aromatic amine stretch); 2972 cm^{-1} (aliphatic C-H stretch); 2361 cm^{-1} (weak N-N=N stretch); 1655 cm^{-1} (aromatic ketone stretch). EI-MS: M^{+}=294.14 amu ($C_{16}H_{14}N_4O_2$); base peak m/z=196 amu ($C_{12}H_6NO_2$).

14.8 Attempted preparation of 7-(diaza-N-benzylpiperazino)-benzopyranopyridine:



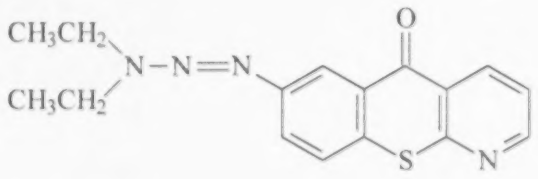
7-Aminobenzopyranopyridine (500 mg, 2.4 mmol) was dissolved in 10 mL 50%^{V}/v H_2SO_4 and cooled in an ice bath to 5-0°C. To this was slowly added 620 mg (9.0 mmol) $NaNO_2$ which had been dissolved in 1.25 mL H_2O. The reaction was stirred in the ice bath for two hours at which time 1.4 mL (1424 mg, 8.0 mmol) N-benzylpiperazine was added slowly. The reaction was allowed to proceed for another 1/2 hour, still in the ice bath, at which time the reaction was basified with 40%^{W}/v NaOH. The resultant precipitate was filtered and the filtrate washed with ethyl acetate. The precipitate was also washed thoroughly with ethyl acetate and filtrate collected. The filtrates and ethyl acetate washes were combined and solvent removed *in vacuo* to leave 295 mg (0.71 mmol) of precipitate with a melting point of 135°C (acetonitrile). ^{1}H NMR and IR were not performed in light of EI-MS results. EI-MS: expected M^{+} of 434 amu was not observed; however, a peak of m/z=196 amu was seen. No desired product was recovered.

14.9 Preparation of 7-(diazadimethylamino)-benzothiopyranopyridine:



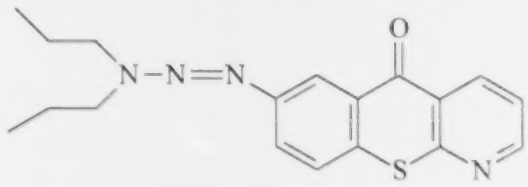
7-Aminobenzothiopyranopyridine (500 mg, 2.19 mmol) was dissolved in 10 mL 50%^{V}/v H_2SO_4 and cooled in an ice bath to 5-0°C. To this was slowly added 620 mg (9.0 mmol) $NaNO_2$ which had been dissolved in 1.25 mL H_2O. The reaction was stirred in the ice bath for two hours at which time 360 mg (8.0 mmol) dimethylamine was added slowly. The reaction was allowed to proceed for another 1/2 hour, still in the ice bath, at which time the reaction was basified with 40%^{W}/v NaOH. The reaction was diluted with 100 mL cold water and 25 mL chloroform and stirred at room temperature for 1 hour. The aqueous layer was then washed with chloroform (4 by 10 mL portions), chloroform layer dried with $MgSO_4$ and solvent removed *in vacuo*. The resultant 115 mg (0.404 mmol) of product had a melting point of 169-171 °C (acetonitrile). ^{1}H NMR ($CDCl_3$): 8.85δ (d, H-2, J=8.2 Hz); 8.79δ (broad s, H-4); 8.60δ (s, H-6); 7.82δ (d, H-8, J=8.3 Hz); 7.61δ (d, H-9, J=8.8 Hz); 7.44δ (dd, H-3, J=4.4, 6.6, 7.2, and 4.9 Hz); 3.53δ (broad s, CH_3); 3.33δ (broad s, CH_3). IR (KBr pellet): 3429 cm^{-1} (aromatic amine stretch); 2898 cm^{-1} (aliphatic C-H stretch); 2368 cm^{-1} (weak N-N=N stretch); 1662 cm^{-1} (aromatic ketone stretch). EI-MS: M^+ =284.07 amu ($C_{14}H_{12}SN_4O$); base peak m/z=212 amu ($C_{12}H_6SNO$).

14.10 Preparation of 7-(diazadiethylamino)-benzothiopyranopyridine:



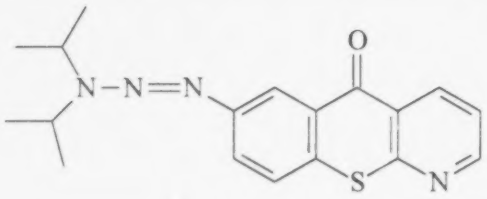
7-Aminobenzothiopyranopyridine (500 mg, 2.19 mmol) was dissolved in 10 mL 50%^{V}/v H_2SO_4 and cooled in an ice bath to 5-0°C. To this was slowly added 620 mg (9.0 mmol) $NaNO_2$ which had been dissolved in 1.25 mL H_2O. The reaction was stirred in the ice bath for two hours at which time 837 µL (584 mg, 8.0 mmol) diethylamine was added slowly. The reaction was allowed to proceed for another 1/2 hour, still in the ice bath, at which time the reaction was basified with 40%^{W}/v NaOH. The reaction was diluted with 100 mL cold water and 25 mL chloroform and stirred at room temperature for 1 hour. The aqueous layer was then washed with chloroform (4 by 10 mL portions), chloroform layer dried with $MgSO_4$ and solvent removed *in vacuo*. The resultant 240 mg (0.77 mmol) of product had a melting point of 101-103°C (acetonitrile). ^{1}H NMR ($CDCl_3$): 8.84δ (dd, H-2, J=1.7, 7.7, 8.3, and 2.2 Hz); 8.79δ (dd, H-4, J=2.2, 4.4, 4.4, and 2.2 Hz); 8.58δ (d, H-6, J=2.8 Hz); 7.82δ (dd, H-8, J=2.8, 8.8, 8.3, and 2.2 Hz); 7.60δ (d, H-9, J=8.8 Hz); 7.44δ (dd, H-3, J=4.4, 8.3, 8.3, and 4.4 Hz); 3.83δ (q, two CH_2, J=7.1, 14.3, 14.3, and 7.1 Hz); 1.21δ (t, two CH_3, J=14.3, and 7.1 Hz). IR (KBr pellet): 3422 cm^{-1} (aromatic amine stretch); 2972 cm^{-1} (aliphatic C-H stretch); 2368 cm^{-1} (N-N=N stretch); 1649 cm^{-1} (aromatic ketone stretch). EI-MS: M^+=312.13 amu ($C_{16}H_{16}SN_4O$); base peak m/z=212 amu ($C_{12}H_6SNO$).

14.11 Preparation of 7-(diazadipropylamino)-benzothiopyranopyridine:



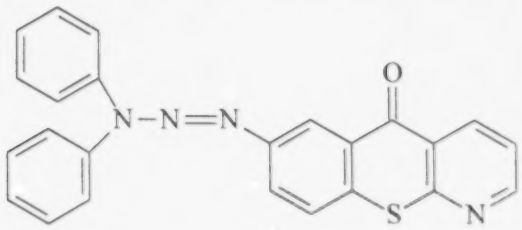
7-Aminobenzothiopyranopyridine (500 mg, 2.19 mmol) was dissolved in 10 mL 50%^{V}/v H_2SO_4 and cooled in an ice bath to 5-0°C. To this was slowly added 620 mg (9.0 mmol) $NaNO_2$ which had been dissolved in 1.25 mL H_2O. The reaction was stirred in the ice bath for two hours at which time 1094 µL (808 mg, 8.0 mmol) dipropylamine was added slowly. The reaction was allowed to proceed for another 1/2 hour, still in the ice bath, at which time the reaction was basified with 40%^{W}/v NaOH. The reaction was diluted with 100 mL cold water and 25 mL chloroform and stirred at room temperature for 1 hour. The aqueous layer was then washed with chloroform (4 by 10 mL portions), chloroform layer dried with $MgSO_4$ and solvent removed *in vacuo*. The resultant 90 mg (0.26 mmol) of product had a melting point of 70°C (acetonitrile). ^{1}H NMR ($CDCl_3$): 8.85δ (dd, H-2, J=1.7, 7.7, 8.3, and 2.2 Hz); 8.79δ (dd, H-4, J=1.7, 4.4, 4.4, and 1.7 Hz); 8.57δ (d, H-6, J=2.2 Hz); 7.81δ (dd, H-8, J=2.7, 8.8, 8.3, and 2.2 Hz); 7.59δ (d, H-9, J=8.79 Hz); 7.43δ (dd, H-3, J=4.4, 7.7, 8.3, and 4.9 Hz); 3.72δ (t, two CH_2, J=7.2, and 6.3 Hz); 0.98δ (t, two CH_3, J=7.1, and 7.7 Hz). IR (KBr pellet): 3429 cm^{-1} (aromatic amine stretch); 3032 cm^{-1} (aromatic C-H stretch); 2965 cm^{-1} (aliphatic C-H stretch); 2368 cm^{-1} (N-N=N stretch); 1649 cm^{-1} (aromatic ketone stretch). EI-MS: M^+=340.08 amu ($C_{18}H_{20}SN_4O$); base peak m/z=212 amu ($C_{12}H_6SNO$).

14.12 Preparation of 7-(diazadi-isopropylamino)-benzothiopyranopyridine:



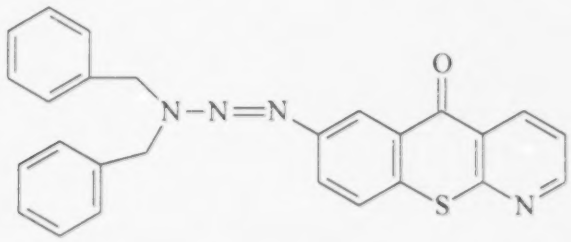
7-Aminobenzothiopyranopyridine (500 mg, 2.19 mmol) was dissolved in 10 mL 50%^{v}/v H_2SO_4 and cooled in an ice bath to 5-0°C. To this was slowly added 620 mg (9.0 mmol) $NaNO_2$ which had been dissolved in 1.25 mL H_2O. The reaction was stirred in the ice bath for two hours at which time 560 µL (4.04 mg, 4.0 mmol) di-isopropylamine was added slowly. The reaction was allowed to proceed for another 1/2 hour, still in the ice bath, at which time the reaction was basified with 40%^{w}/v NaOH. The reaction was diluted with 100 mL cold water and 25 mL chloroform and stirred at room temperature for 1 hour. The aqueous layer was then washed with chloroform (4 by 10 mL portions), chloroform layer dried with $MgSO_4$ and solvent removed *in vacuo*. The resultant 119 mg (0.35 mmol) of product had a melting point of 182°C (acetonitrile). ^{1}H NMR ($CDCl_3$):8.85δ (m, H-2 and H-4 overlapping, J=2.8 and 6.6 Hz); 8.54δ (d, H-6, J=2.7 Hz); 8.45δ (dd, H-8, J=2.8, 8.8, 8.8, and 2.8 Hz); 7.82δ (d, H-9, J=8.8 Hz); 7.43δ (dd, H-3, J=2.8, 7.7, 8.2, and 3.3 Hz); 1.41δ (broad s, CH); 1.32δ (broad s, CH_3). IR (KBr pellet): 3429 cm^{-1} (aromatic amine stretch); 3032 cm^{-1} (aromatic C-H stretch); 2972 cm^{-1} (aliphatic C-H stretch); 2368 cm^{-1} (N-N=N stretch); 1655 cm^{-1} (aromatic ketone stretch). EI-MS: M^+=340.15 amu ($C_{18}H_{20}SN_4O$); base peak m/z=212 amu ($C_{12}H_6SNO$).

14.13 Attempted preparation of 7-(diazadiphenylamino)-benzothiopyranopyridine:



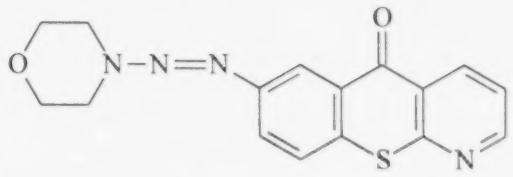
7-Aminobenzothiopyranopyridine (500 mg, 2.19 mmol) was dissolved in 10 mL 50%^{V}/v H_2SO_4 and cooled in an ice bath to 5-0°C. To this was slowly added 620 mg (9.0 mmol) $NaNO_2$ which had been dissolved in 1.25 mL H_2O. The reaction was stirred in the ice bath for two hours at which time 700 mg (4.14 mmol) diphenylamine was added slowly. The reaction was allowed to proceed for another 1/2 hour, still in the ice bath, at which time the reaction was basified with 40%^{W}/v NaOH. The reaction was diluted with 100 mL cold water and 25 mL chloroform and stirred at room temperature for 1 hour. The aqueous layer was then washed with chloroform (4 by 10 mL portions), chloroform layer dried with $MgSO_4$ and solvent removed *in vacuo*. The resultant oil was purified from acetonitrile but the desired product could not be isolated from the black oil.

14.14 Attempted preparation of 7-(diazadibenzylamino)-benzothiopyranopyridine:



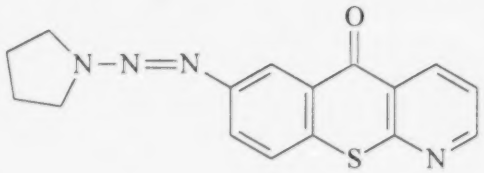
7-Aminobenzothiopyranopyridine (500 mg, 2.19 mmol) was dissolved in 10 mL 50%^{V}/v H_2SO_4 and cooled in an ice bath to 5-0°C. To this was slowly added 620 mg (9.0 mmol) $NaNO_2$ which had been dissolved in 1.25 mL H_2O. The reaction was stirred in the ice bath for two hours at which time 768 μL (788 mg, 4.0 mmol) dibenzylamine was added slowly. The reaction was allowed to proceed for another 1/2 hour, still in the ice bath, at which time the reaction was basified with 40%^{W}/v NaOH. The reaction was diluted with 100 mL cold water and 25 mL chloroform and stirred at room temperature for 1 hour. The aqueous layer was then washed with chloroform (4 by 10 mL portions), chloroform layer dried with $MgSO_4$ and solvent removed *in vacuo*. The resultant 345 mg (0.79 mmol) of product had a melting point of 197°C (acetonitrile). ^{1}H NMR ($CDCl_3$): 9.37δ (broad s); 7.35δ (m, J= 3.3, 3.8, and 4.4 Hz); 7.28δ (m, J=3.3, 3.3, and 3.8 Hz); 4.00δ (s). IR (KBr pellet): 3422 cm^{-1} (aromatic amine stretch); 3040 cm^{-1} (aromatic C-H stretch); 2985 cm^{-1} (aliphatic C-H stretch); ketone peak missing; 1383 cm^{-1} (aromatic C-N stretch). EI-MS: expected $M^{+\cdot}$=436 amu was not observed, nor was m/z=212 amu. Observed was $M^{+\cdot}$=197 amu ($C_{14}H_{15}N$), and m/z=91 amu (C_7H_7, tropylium ion). Product is not the one desired.

14.15 Preparation of 7-(diazamorpholino)-benzothiopyranopyridine:



7-Aminobenzothiopyranopyridine (500 mg, 2.19 mmol) was dissolved in 10 mL 50%^{v}/v H_2SO_4 and cooled in an ice bath to 5-0°C. To this was slowly added 620 mg (9.0 mmol) $NaNO_2$ which had been dissolved in 1.25 mL H_2O. The reaction was stirred in the ice bath for two hours at which time 345 µL (348 mg, 4.0 mmol) morpholine was added slowly. The reaction was allowed to proceed for another 1/2 hour, still in the ice bath, at which time the reaction was basified with 40%^{w}/v NaOH. The reaction was diluted with 100 mL cold water and 25 mL chloroform and stirred at room temperature for 1 hour. The aqueous layer was then washed with chloroform (4 by 10 mL portions), chloroform layer dried with $MgSO_4$ and solvent removed *in vacuo*. The resultant 175 mg (0.54 mmol) of product had a melting point of 205-207°C (acetonitrile). ^{1}H NMR ($CDCl_3$): 8.84δ (dd, H-2, J=1.7, 7.7, 8.3, and 2.2 Hz); 8.79δ (dd, H-4, J=2.2, 4.4, 4.4, and 2.2 Hz); 8.62 δ (d, H-6, J=2.2); 7.82δ (dd, H-8, J=2.2, 8.3, 8.3, and 2.2 Hz); 7.63δ (d, H-9, J=8.8 Hz); 7.44δ (dd, H-3, J=4.4, 8.3, 8.3, and 4.4 Hz); 3.88δ (s, morpholino H's). IR (KBr pellet): 3429 cm^{-1} (aromatic amine stretch); 3050 cm^{-1} (aromatic C-H stretch); 2958 cm^{-1} (aliphatic C-H stretch); 2200-2400 cm^{-1} N-N=N stretch missing; 1642 cm^{-1} (aromatic ketone stretch). EI-MS: M^+=326.11 amu ($C_{16}H_{14}SN_4O_2$); base peak m/z=212 amu ($C_{12}H_6SNO$).

14.16 Preparation of 7-(diazapyrrolidino)-benzothiopyranopyridine:



7-Aminobenzothiopyranopyridine (500 mg, 2.19 mmol) was dissolved in 10 mL 50%^{V}/v H_2SO_4 and cooled in an ice bath to 5-0°C. To this was slowly added 620 mg (9.0 mmol) $NaNO_2$ which had been dissolved in 1.25 mL H_2O. The reaction was stirred in the ice bath for two hours at which time 333 µL (284 mg, 4.0 mmol) pyrrolidine was added slowly. The reaction was allowed to proceed for another 1/2 hour, still in the ice bath, at which time the reaction was basified with 40%^{W}/v NaOH. The reaction was diluted with 100 mL cold water and 25 mL chloroform and stirred at room temperature for 1 hour. The aqueous layer was then washed with chloroform (4 by 10 mL portions), chloroform layer dried with $MgSO_4$ and solvent removed *in vacuo*. The resultant 390 mg (1.26 mmol) of product had a melting point of 172-173°C (acetonitrile). ^{1}H NMR ($CDCl_3$): 8.84δ (dd, H-2, J=2.28.3, 7.7, and 1.7 Hz); 8.78δ (dd, H-4, J=1.74.4, 4.4, and 1.7 Hz); 8.58δ (d, H-6, J=2.2 Hz); 7.80δ (dd, H-8, J=2.7, 8.8, 8.3, and 2.2 Hz); 7.59δ (d, H-9, J=8.3 Hz); 7.43δ (dd, H-3, J=4.4, 7.7, 8.3, and 4.9 Hz); 3.95δ (broad s, H-1' and H-4'); 3.74δ (broad s, H-1' and H-4'); 2.06δ (s, H-2' and H-3'); 2.01δ (s, H-2' and H-3'). IR (KBr pellet): 3435 cm^{-1} (aromatic amine stretch); 3059 cm^{-1} (aromatic C-H stretch); 2980 cm^{-1} (aliphatic C-H stretch); 2368 cm^{-1} (N-N=N stretch); 1642 cm^{-1} (aromatic ketone stretch). EI-MS: M^+=310.13 amu ($C_{16}H_{14}SN_4O$); base peak m/z=212 amu ($C_{12}H_6SNO$).

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